EFFECT OF CHROMIUM ON THE GROWTH OF UNICELLULAR CHLOROPHYCEAE AND DIATOMS

RALPH J. HERVEY[1]

Introduction

In attempts to isolate and culture some of the more abundant algae occurring in the waters of the Bear River Migratory Bird Refuge in Utah, difficulty in growing certain ones was encountered. The presence of copper in the distilled water was at first suspected as the cause of failure, but media prepared from glass-distilled water gave no better results. Because glassware for all cultural studies was cleaned in dichromate cleaning solution, and because chromium in minute concentration is known to be toxic to many microörganisms, inhibition by this metal was suspected. Since little is known specifically concerning the effect of chromium on algal growth, a series of experiments was performed, designed to determine the sensitivity of some representative algae to this heavy metal.

The necessity of using chemically clean glassware for culturing algae has long been emphasized by phycologists (1, 3, 9). The most common laboratory method for obtaining clean glassware employs an acid-dichromate cleaner. Recent studies, however, have indicated that it is difficult to wash completely from glass surfaces both H and Cr_2O_7 ions; consequently, that cleaning procedure is open to criticism in microbiological investigations. LAUG (7) found that ten rinsings did not suffice completely to remove Cr_2O_7 from glass, since apparently dichromate-free surfaces, if allowed contact with water for several hours, yielded detectable Cr_2O_7 in solution. RICHARDS (10) studied the effects of traces of Cr_2O_7 on yeast and several algal species and found that as little as 0.1–1.0 p.p.m. was toxic. HENRY and SMITH (5) have recently confirmed the findings of LAUG and RICHARDS, showing that after the tenth rinsing a dichromate-cleaned pipette yielded between 0.1 and 0.2 p.p.m. of Cr_2O_7.

The presence of toxic substances in trace amounts may assume the greatest significance in dilute synthetic media, such as those commonly used for culturing algae, inasmuch as such substrates lack the protective effect afforded by proteins or other large organic molecules generally composing bacterial or fungal media. Thus, HENRY and SMITH (5) found that 1 p.p.m. of Cr_2O_7 in a synthetic medium was very toxic to *Staphylococcus aureus* but that approximately ten times as much was required to produce equivalent growth inhibition in beef extract-peptone broth.

While chromium is toxic to many microörganisms, it stimulates the metabolism of others. Although BRENCHLEY (2), in her recent review, did not include Cr as an element essential to plant nutrition, PRATT and DUFRENOY (8) reported stimulation by Cr of penicillin production by *Penicillium chrysogenum*. One hundred p.p.m. of Cr as Cr_2O_7 stimulated maximum yield, while 200 p.p.m. permitted good growth. Virtually no antibiotic was produced in the absence of

[1] Formerly bacteriologist, Bear River Research Station, Fish and Wildlife Service, Brigham, Utah.

Cr. Stimulating or inhibiting effects of Cr appear to be exerted through its action on enzyme systems. HORECKER *et al.* (6) found that both aluminum and chromium stimulated the succinic dehydrogenase-cytochrome system, the latter being most active. HENRY and SMITH (5), on the contrary, found that urease activity was almost completely inhibited by 1–10 p.p.m. of Cr_2O_7.

Material and methods

Three groups of unicellular algae, namely, the Chlorococcales, Euglenaceae, and Diatomaceae, represented by the following species and unidentified forms: *Chlorella variegatus, Chlorococcum humicola, Scenedesmus obliquus; Lepocinclis steinii*, green flagellate no. 46; and two diatoms, no. 26 and no. 47 belonging to the order Pennales, were selected for study. All are uni-algal cultures containing one or more types of bacterial contaminants, except no. 47, which is a pure culture. Isolation of all organisms was made by means of an agar-plating procedure.

Two liquid media, H-2 and H-2 modified, supplied the basal nutrients. Previous study had shown these media to be very satisfactory for culturing many algal organisms; forty-three uni-algal cultures, including greens, blue-greens, and diatoms, had been successfully grown on them. Medium H-2 is a dilute, synthetic, entirely inorganic solution and is derived in part from formulas suggested by CHU (3, 4). Medium H-2 modified is nonsynthetic, containing soil extract. The formula for H-2 synthetic medium is as shown in the next column. Medium H-2 modified is prepared by mixing 600 ml. of H-2 with 400 ml. of soil extract made from river water.

A series of ten solutions containing the following concentrations of chromium as Cr_2O_7: 32, 16, 6.4, 3.2, 1.6, 0.32, 0.032, 0.0032, 0.00032, and 0.00011 p.p.m. was prepared from each of the two basal media, H-2 and H-2 modified, by diluting a stock solution of C.P. $K_2Cr_2O_7$ (1 gm./100 ml. of distilled water) in calculated volumes of each of the above solutions. The resulting media, containing graded concentrations of chromium as Cr_2O_7, were distributed in 4-ml. quantities in 18 × 125-mm. Pyrex test tubes, which were then plugged with cotton and sterilized in an autoclave. Because it was desired to avoid Cr_2O_7 contamination to the glassware, the tubes were thoroughly washed in a hot detergent solution and carefully rinsed several times in distilled water before use.

Constituents	Concentration (p.p.m.)
KNO_3	400
K_2HPO_4	50
$MgSO_4 \cdot 7H_2O$	250
$CaCl_2 \cdot 2H_2O$	20
K_2CO_3	500
$MnSO_4 \cdot 4H_2O$	15
$Fe(NH_4)_2(SO_4)_2 \cdot 4H_2O$	20
$NaSiO_3 \cdot 9H_2O$	500
KI	10
Distilled water	1000 ml.

Duplicate tubes of each dichromate medium were inoculated with a standard loop-full from vigorous cultures of each of the algal species employed, the order of inoculation being arranged so that the media having the greatest concentration of Cr were seeded last to prevent contamination of the controls (basal media containing no Cr) and less concentrated media. The tubes were incubated upright in open wire racks located near a large window on the north side of the building, thus avoiding exposure to the direct rays of the sun. The racks were turned daily to insure maximum illumination of all tubes. The incubation temperature varied between 15° and 20° C.

Since a photoelectric colorimeter was not available, quantity of growth was estimated visually after 15, 33, and 56 days of incubation. Although most of the species yielded homogeneous cellular suspensions when the culture tubes were agitated, thus making estimation of comparative growth relatively easy, the diatoms tended to form large adherent masses of cells which made accurate estimation difficult. The numbers 1, 2, 3, 4, 5, 6 or fractions of these appearing in the tables and figures designate the quantity of growth produced and are averages obtained by reading duplicate culture tubes. The growth represented by each number is as follows: 0, no growth; 1, small amount of growth; 2, fair growth; 3, good growth; 4, excellent growth; 5, abundant growth; and 6, very abundant growth.

TABLE 1

GROWTH OF FOUR NONFILAMENTOUS GREEN ALGAE AND TWO DIATOMS IN SYNTHETIC MINERAL MEDIUM, H-2, AND IN MEDIUM H-2 MODIFIED WITH SOIL EXTRACT, BOTH CONTAINING GRADED CONCENTRATIONS OF CHROMIUM

	H-2			H-2 MODIFIED			H-2			H-2 MODIFIED		
CR P.P.M.	15 days	33 days	56 days	15 days	33 days	56 days	15 days	33 days	56 days	15 days	33 days	56 days
	Chlorella variegatus						Chlorococcum humicola					
0.0	3	5	5	3	4	5	1.5	4	4.5	3.5	5	4.5
.0001	2.5	5	5	2	3.5	5	3	5	4	2	4	4.5
.0003	2	4.5	5	2.5	3.5	5	3	4.5	4.5	1	4	4
.0032	2	4.5	5	2	3.5	5	3	4.5	5	1	3.5	4
.032	2	5	5	2	3	5	3	5	4.5	1.5	3.5	4
0.32	2	5	5	2	3.5	5	2.5	4	4.5	2	4	4
1.0	1	2.5	5	1	2.5	5	0.5	1.5	3	1.5	2.5	3
3.2	0	0	3.5	0	0.5	4.5	0	1.5	2	0.5	1.5	2.5
6.4	0	0.1	3	0	0	2	0	0	0	0	0	0
16.0	0	0	0	0	0	0	0	0	0	0	0	0
	Flagellate # 46						Lepocinclis steinii					
0.0	2	5	5.5	2	4.5	6	4	4	4	4.5	4.5	4.5
.0001	4	5.5	5.5	1	4	6	3	3.5	4	3.5	4.5	4.5
.0003	3	5	5.5	1	4	6	3	3.5	4	3	4	4.5
.0032	2	5.5	5.5	1	4	6	4.5	4.5	4	3.5	4	4.5
.032	1.5	5	5.5	1	4	5.5	5	4	4.5	4	5	5
0.32	0.1	3.5	5.5	0	3.5	5.5	0	1	4	0	2.5	5
1.0	0	0	0	0	0	3	0	0	0	0	0	0
	Diatom # 47						Diatom # 26					
0.0	0	2	4.5	3	3.5	5.5	0	0.1	2	3.5	4	4
.0001	0	3	4	1	3	5	0	2.5	2.5	1.5	4	4.5
.0003	0	2	4	2	2.5	4	0	0.1	1	2.5	4	4
.0032	0	2	4.5	2.5	2	4.5	0	1	1.5	2	4	4
.032	0	2	4.5	3	3	4.5	0.1	3	2.5	3	4	3.5
0.32	0	0	0	0	0.1	4	0	0.1	2.5	2	4	4

Results and discussion

For convenience of discussion the experimental data are considered under several headings, based on growth response to different concentrations of chromium. Before entering into a discussion of the results under each of the categories, a few explanatory statements are necessary. A summary of all growth data for six of the seven algae is presented in table 1. Data for *Scenedesmus obliquus* have been omitted, because its response to Cr is very similar to that of *Chlorococcum humicola*. Some of the data for two species, *C. variegatus* and diatom # 47, derived from table 1, are also graphically presented in figures 1 and 2. Only those concentrations of Cr which caused differences within the graded series of Cr concentrations or between themselves and the controls have been graphed. Thus, wherever a series of concentrations showed no difference within the group, only one representative value has been graphed. Omission of a value for a given medium, as for the 15-day incubation period in figure 2, indicates no growth.

Concentrations of chromium completely inhibiting growth

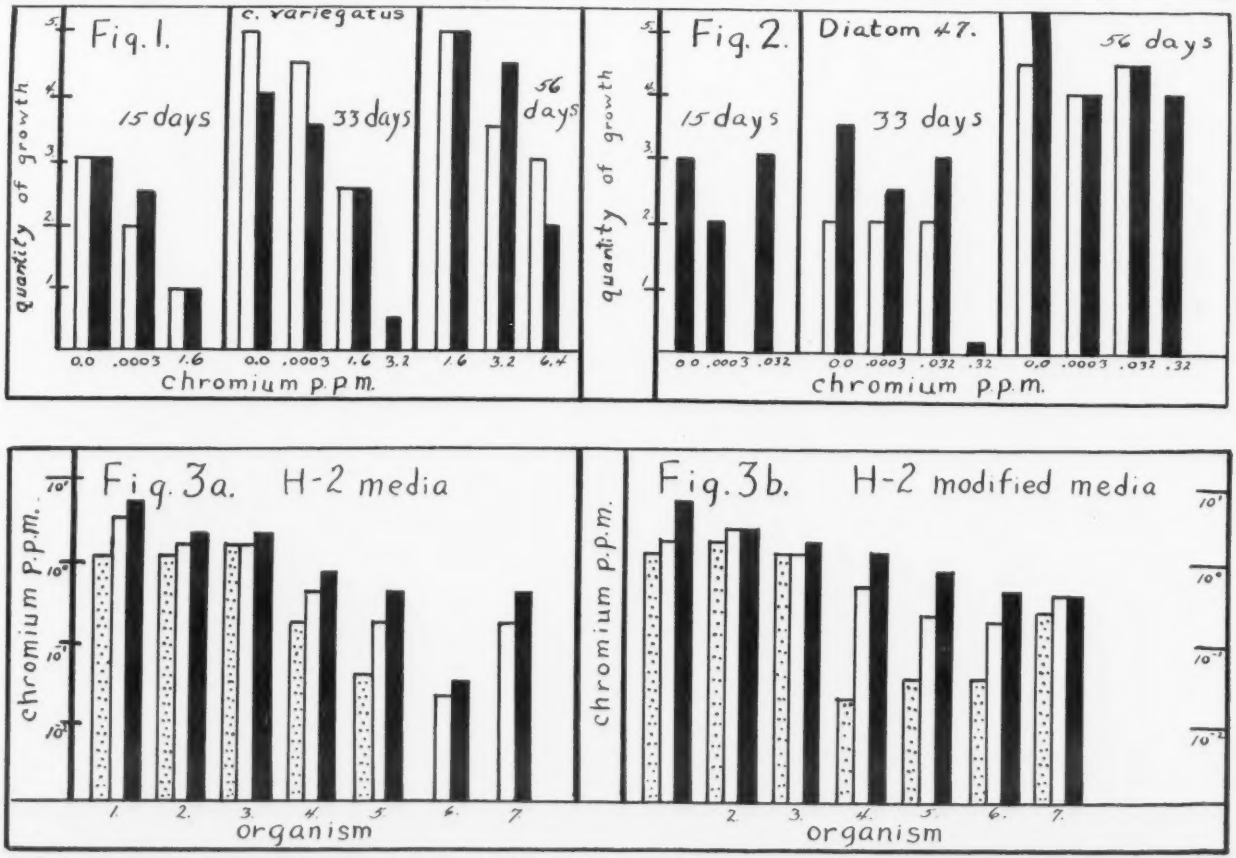
On the basis of the species studied, it is apparent that at least some algae, i.e., the diatoms, are as sensitive to chromium as are many yeasts and bacteria, because a few hundredths of a microgram per milliliter completely suppressed their growth (table 2). Certain others, namely, *C. variegatus*, *C. humicola*, and *S. obliquus*, all belonging to the Chlorococcales, are relatively tolerant to Cr. Intermediate between the tolerant and the sensitive groups are the two euglenoid flagellates.

Growth of diatom # 47, the most sensitive of the two studied, was inhibited completely by 0.32 p.p.m. of Cr in synthetic medium, while a concentration of 1.6 p.p.m. was completely inhibitory in the medium containing soil extract. With 0.32 p.p.m. in the latter medium, excellent growth developed after 56 days of incubation. Diatom # 26 was somewhat less sensitive but more irregular in growth than # 47 in H-2 medium. In H-2 modified medium, # 26's sensitivity to Cr was five- to tenfold less than that of # 47 up to 33 days of growth, but after 56 days the tolerance of the two organisms was about equal in the two media.

For the two euglenoids the limiting concentrations of Cr were somewhat greater than those for the diatoms. Growth of both flagellates, *L. steinii* and # 46, was completely suppressed in concentrations ranging between 0.32 and 1.6 p.p.m. in H-2 medium. In H-2 modified medium flagellate # 46 was able to tolerate more than 1.6 p.p.m. but only after a prolonged incubation period.

In contrast to the sensitivity of the four organisms just discussed, the three members of the Chlorococcales were relatively much less sensitive to Cr. *C. variegatus*, the most tolerant of the group, was completely suppressed by approximately 3.2 p.p.m. of Cr over a period of more than 33 days, regardless of the nature of the basal medium. After prolonged incubation the organism was able to tolerate more than 6.4 p.p.m. *S. obliquus* and *C. humicola*, although not able to grow in concentrations of Cr much above 3.2 p.p.m., initiated growth in equal or greater concentrations than did *C. variegatus*.

In considering the growth-limiting concentrations of Cr for the seven organisms studied, it should be pointed out that, since the algae grow slowly, growth was necessarily observed over a relatively long period of time (56 days). Appre-



FIGS. 1–3.—Figs. 1–2, growth in several concentrations of chromium on two different basal media, H-2 (*white bars*) and H-2 modified (*black bars*); fig. 1, *Chlorella variegatus;* fig. 2, diatom #47. Fig. 3, approximate highest concentrations of chromium allowing traces of growth for seven algae (1, *C. variegatus;* 2, *C. humicola;* 3, *S. obliquus;* 4, flagellate #46; 5, *L. steinii;* 6, diatom #47; 7, diatom #26). After incubation for 15 days (*dotted bars*), 33 days (*white bars*), and 56 days (*black bars*).

ciable evaporation, therefore, occurred in the culture media. Because there was as much as 50% reduction in volume over the 56-day period, the growth-limiting concentrations of Cr are actually somewhat greater than the tabulated values indicate.

Concentrations of chromium which allow growth

In the previous section the approximate ranges of chromium concentrations which completely limited growth of seven species of algae were presented (table 2). Somewhere within these concentration ranges the organisms could also initiate slight growth. Those concentrations which allowed at least some growth, i.e., threshold concentrations, are graphically presented in figure 3. These approximate values were estimated from the data in table 1 by combining concentrations and growth values. A given threshold-concentration value was increased over the corresponding value in table 1 in proportion to the quantity of growth made. These near-growth-limiting concentrations generally are considerably higher than the concentrations which allowed equal or better growth than the controls (table 3). Between these two concentration values, the quantity of growth varied inversely with the Cr concentration (figs. 1, 2, table 1). The greatest concentrations of Cr which allowed growth equal, or superior, to the controls at various periods during incubation are shown in table 3.

To show the relationship between threshold concentration and optimal-growth concentration for each species, ratios have been calculated by dividing threshold-growth concentration values by optimal-growth concentration values (table 4). These ratios, relatively wide in the early periods of growth, tend gradually to narrow down to unity. This tendency, which is especially marked in *C. variegatus*, would seem to indicate that the organisms slowly adapt themselves to chromium. A ratio of 1 probably indicates that the cell's adaptive processes are unable to accommodate the organism to higher concentrations of the heavy metal, at least over a period of 56 days. When the ratios are greater than unity, the organism is apparently potentially able to adjust itself to still higher concentrations of Cr. Thus the three members of the Chlorococcales appear to

TABLE 2

Approximate concentration ranges of chromium, p.p.m., which completely inhibited growth in seven species of algae

Organism	H-2			H-2 modified		
	15 days	33 days	56 days	15 days	33 days	56 days
C. variegatus	1.6 –3.2	1.6 –3.2	6.4 –16.0	1.6 –3.2	3.2 –6.4	6.4 –16.0
C. humicola	1.6 –3.2	3.2 –6.4	3.2 – 6.4	3.2 –6.4	3.2 –6.4	3.2 – 6.4
S. obliquus	3.2 –6.4	3.2 –6.4	3.2 – 6.4	1.6 –3.2	1.6 –3.2	1.6 – 3.2
L. steinii	0.032–0.32	0.32 –1.6	0.32 – 1.6	0.032–0.32	0.32 –1.6	0.32 – 1.6
Flagellate #46	0.032–0.32	0.32 –1.6	0.32 – 1.6	0.032–0.32	0.32 –1.6	1.6 – 3.2
Diatom #26	No growth*	0.032–0.32	0.32 – 1.6	0.32 –1.6	0.32 –1.6	0.32 – 1.6
Diatom #47	No growth*	0.032–0.32	0.032– 0.32	0.032–0.32	0.032–0.32	0.32 – 1.6

* The lag period for both these organisms was so extended in H-2 medium that even the controls showed no growth in 15 days.

possess adaptive potentialities superior to those of the flagellates and diatoms. *C. variegatus* would appear to possess an especially rapid adaptive ability.

On the basis of absolute tolerance to Cr, i.e., ability to make at least some growth, the seven species studied may be grouped as "least," "intermediately," and "most sensitive." This grouping, however, is not possible when considering concentrations which allow optimal growth. Those organisms in the top half of table 2, which on the basis of threshold-growth concentrations are many times more tolerant than those occupying the lower half of that table, are, on the basis of optimum growth, little more tolerant toward Cr than the bottom group. In fact, *C. variegatus*, previously indicated to be the most tolerant organism to Cr, was unable to duplicate control growth during the first 15 days in either basal medium containing over 0.00032 p.p.m. By contrast, diatom # 26, a very sensitive organism on the basis of the earlier grouping, was able to make optimal growth in a hundred-fold greater concentration than was *C. variegatus*.

TABLE 3

GREATEST CONCENTRATION OF CHROMIUM, P.P.M., WHICH PERMITTED GROWTH EQUAL TO, OR BETTER THAN, CONTROLS (NO CR) IN SEVEN ALGAL SPECIES

ORGANISM	H-2			H-2 MODIFIED		
	15 days	33 days	56 days	15 days	33 days	56 days
C. variegatus	0.0001	0.32	1.6	0.00032	0.32	3.2
C. humicola	.32	.32	0.32	.0	.32	0.32
S. obliquus	.32	.32	.32	.0	.32	.32
L. steinii	.032	.032	.32	.032	.032	.32
Flagellate #46	0.032	.032	.32	.0	.032	.32
Diatom #26	*	.32	.32	.032	.32	.32
Diatom #47	*	0.032	0.032	0.032	0.032	0.32

* No growth in any tubes, including controls.

TABLE 4

RATIOS OF CONCENTRATION OF CHROMIUM ALLOWING TRACE GROWTH TO CONCENTRATION WHICH PERMITTED OPTIMAL GROWTH IN SEVEN ALGAL SPECIES

ORGANISM	H-2			H-2 MODIFIED		
	15 days	33 days	56 days	15 days	33 days	56 days
C. variegatus	1454	20	4	500	10	2
C. humicola	5	10	10	*	10	10
S. obliquus	10	10	10	.....	5	5
L. steinii	1	5	1	1	5	1
Flagellate #46	10	10	1	.....	10	5
Diatom #26	*	10	1	10	1	1
Diatom #47	*	1	1	1	10	1

* None of cultures containing Cr produced growth equal to control during this early period, thus making calculation of a ratio impossible.

After 56 days of growth, *C. variegatus* and diatom # 47 were the only organisms that could be distinguished from the other species on the basis of greater or lesser tolerance for Cr.

Growth-stimulating concentrations of chromium

In the two previous sections those concentrations of the heavy metal which completely inhibited growth, as well as those which allowed it, have been indicated and discussed. In addition, as with many toxic substances, chromium, when present in sublethal quantities, stimulated growth in certain of the algal species but not in others (table 5). Stimulation, when it occurred, generally was found in the synthetic medium. *C. variegatus*, the most tolerant and rapidly adaptive organism to Cr, nevertheless, was not stimulated by its presence. All other species were stimulated to some extent. Certain species were stimulated over a relatively wide concentration, e.g., *C. humicola*, 0.0001–0.32 p.p.m. As a general rule, also, stimulation occurred only in the earlier incubation periods, no effect being apparent after the culture aged. *L. steinii*, however, differed from the other species in two ways. Its growth was stimulated by Cr in the nonsynthetic medium, and better than optimal growth continued in both basal media beyond 56 days of growth.

TABLE 5

Concentration ranges of chromium, p.p.m., which stimulated growth in seven species of algae

Organism	H-2			H-2 modified		
	15 days	33 days	56 days	15 days	33 days	56 days
C. variegatus	None	None	None	None	None	None
C. humicola	0.0001 –0.32	0.0001 –0.32	None	None	None	None
S. obliquus	0.0001 –0.32	0.00032–0.32	None	None	None	None
L. steinii	0.00032–0.32	0.00032–0.32	0.32	None	0.32	0.32
Flagellate #46	0.0001 –0.00032	0.0001 –0.0032	None	None	None	None
Diatom #26		0.0001 –0.32	None	None	None	None
Diatom #47		0.0001 –0.00032	None	None	None	None

Sensitivity to chromium as influenced by composition of nutrient medium

It has already been pointed out that the various species responded differently to Cr in the two basal media employed. These media of different composition were purposely used, because two factors appeared to be present which might possibly modify the toxicity of chromium. These factors are: (*a*) the vigor of growth permitted by a given nutrient medium and (*b*) the protective effect afforded by the constituents of a medium. Preliminary studies indicated that, although the seven species grew satisfactorily on both media, certain species were favored by one or the other substrate. Consequently, it was thought that the ability to make better growth on a certain medium would also be reflected in increased tolerance to Cr. In addition, because of the composition of the H-2 modified medium which contains organic substances, it appeared that a protective effect against metal toxicity might be expected.

Reference to the controls in table 1 will show the favoring effect of one medium over another on the growth of the organisms. This effect, while somewhat apparent in the five members of the Chlorophyceae studied, was especially marked with the two diatoms. The latter were definitely favored by the medium containing soil extract. Both of these organisms exhibited a lag period of over 15 days in the synthetic medium, whereas good growth was attained after 15 days in the nonsynthetic medium. This favoring effect continued throughout the 56 days of growth for the diatoms. *C. humicola*, by contrast, was favored only temporarily by soil extract, since after 33 days the quantity of growth was nearly equivalent in the two media. For the other green forms the two media were about equally satisfactory.

Although preliminary studies had indicated the superiority of the nonsynthetic medium for certain of the algae, it remained to be determined how this enhanced growth would be reflected in terms of tolerance for Cr. In discussing this differential effect, it is convenient to consider tolerance to Cr under the same categories as those used previously for section headings, i.e., concentrations completely suppressing growth, concentrations allowing some growth, etc.

Reference to tables 1 and 2 and figure 3 will show the influence of the two media on the toxicity of Cr in the concentration zones of complete or nearly complete suppression of growth.

In general, the algae were able to make at least slight growth in higher concentrations of Cr in the medium containing soil extract (table 2). An exception to this trend is *S. obliquus*, which tolerated more Cr on H-2 medium. The two diatoms best illustrate the salutary effects of soil extract; slight growth occurred in two- to tenfold greater concentrations of the heavy metal in H-2 modified medium than in H-2. Thus it appears that media which allow superior growth of an organism also endow it with increased tolerance to chromium.

If that series of concentrations which permitted optimum growth (table 3) is next considered in relation to type of basal medium, a somewhat different relationship exists from that just indicated. The diatoms which were definitely favored by H-2 modified now seem less completely favored by it. *C. variegatus* was able to develop optimally in twice the concentration of Cr when the basal medium contained soil extract, but the other four green algae tolerated more Cr in the early periods of growth on H-2 medium. Later, however, they showed about equal tolerance on the two media.

It was previously indicated that the stimulating effect of Cr on growth occurred almost exclusively on the synthetic medium. Although the cause for this phenomenon has not been determined, it would seem likely that there were substances in the medium containing soil extract which are themselves stimulating and which, therefore, may have masked the potential stimulation by Cr. It is also possible that substances, either organic or inorganic, present in the soil extract, partly removed the Cr by adsorption or precipitation.

As a result of the differing tolerances to chromium exhibited by the several organisms, depending on the nature of the medium, it appears that the degree of growth vigor induced by a basal medium is an important factor in increasing tolerance to the heavy metal. It was not possible, however, to demonstrate a protective effect in either of the two media employed, in spite of the presence of organic substances in one of them. An or-

ganism like diatom # 26 is more tolerant to Cr in H-2 modified medium than in H-2 medium largely because the nonsynthetic solution is a more suitable nutrient medium for that organism.

The results obtained with the seven species of unicellular algae studied indicate a wide range in the sensitivity of this group of plants to the heavy metal, chromium. Some of the green forms, especially those belonging to the Chlorococcales, are relatively tolerant to Cr; but other green forms, such as the euglenoid flagellates, are much more sensitive to it. The diatoms are very sensitive to traces of the metal and would very likely fail to grow in many of the media proposed for their isolation and cultivation if traces of Cr_2O_7 ion were to contaminate the substrate. The inhibition of growth would be especially likely under conditions of light inoculation such as occur when dilution cultures are prepared for plate cultures. It is very likely that even less sensitive organisms would also fail to develop where only a few cells composed the inoculum.

The use of dichromate-sulfuric acid cleaning solution would thus seem to be strictly precluded in any investigations involving the isolation and cultivation of algae. Because of the extreme sensitivity of many forms to Cr and because it is practically impossible completely to remove adsorbed Cr_2O_7 ions from glassware exposed to such a cleaning agent, more reliable results may be expected when glassware is cleaned with detergent or nitric acid cleaners.

Summary

1. Seven species of Chlorophyceae and diatoms were cultured over a period of 56 days in two different basal media in the presence of graded concentrations of chromium ranging from 0.0001 to 32 p.p.m.

2. These algae, on the basis of the concentrations of Cr required for complete suppression of growth, fell into three groups as follows: (*a*) most tolerant, three members of the Chlorococcales, 3.2–6.4 p.p.m. of Cr; (*b*) intermediately tolerant, two euglenoid flagellates, 0.32–1.6 p.p.m.; (*c*) least tolerant, two diatoms, 0.032–0.32 p.p.m.

3. The organisms, on the basis of concentrations of Cr allowing optimum growth, showed greater similarity in sensitivity to Cr, with five of the seven species being unable to develop optimally in concentrations above 0.32 p.p.m. *Chlorella variegatus* grew normally in 1.6–3.2 p.p.m., while diatom # 47 could tolerate no more than 0.032–0.32 p.p.m., depending on the basal medium.

4. Chromium in sublethal concentrations stimulated the growth of several of the organisms, especially on the synthetic, inorganic medium.

5. The composition of the basal media, one synthetic, the other similar but also containing soil extract, influenced both the growth vigor and the sensitivity of the organisms to Cr. Growth vigor and degree of tolerance for Cr were positively correlated.

BLACKLAND EXPERIMENT STATION
TEMPLE, TEXAS

LITERATURE CITED

1. BOLD, H. C. The cultivation of algae. Bot. Rev. **8**:69–138. 1942
2. BRENCHLEY, W. E. The essential nature of certain minor elements for plant nutrition. II. Bot. Rev. **13**:169–193. 1947.
3. CHU, S. P. The influence of the mineral composition of the medium on the growth of planktonic algae. I. Methods and culture media. Jour. Ecology **30**:284–325. 1943.
4. ———. The influence of the mineral composition of the medium on the growth of planktonic algae. II. The influence of the concentration of

inorganic nitrogen and phosphate phosphorus. Jour. Ecology **31**:109–148. 1943.

5. HENRY, R. J., and SMITH, E. C. Use of sulfuric acid-dichromate mixture in cleaning glassware. Science **104**:426–427. 1946.
6. HORECKER, B. L.; STOTZ, E., and HOGNESS, T. R. The promoting effect of aluminum, chromium, and the rare earths in the succinic dehydrogenase-cytochrome system. Jour. Biol. Chem. **128**:251–256. 1939.
7. LAUG, E. P. Retention of dichromate by glassware after exposure to potassium dichromate cleaning solution. Ind. Eng. Chem. (anal. ed.), **6**:111–112. 1934.
8. PRATT, R., and DUFRENOY, J. Comments by readers. Science **105**:574. 1947.
9. PRINGSHEIM, E. G. Pure Cultures of Algae: Their Preparation and Maintenance. Macmillan Co., New York. 1946.
10. RICHARDS, O. W. Killing organisms with chromium as from incompletely washed bichromate-sulfuric acid cleaned glassware. Physiol. Zoöl. **9**:246–253. 1936.

VELOCITIES OF WATER ABSORPTION BY INDIVIDUAL ROOT HAIRS OF DIFFERENT SPECIES

H. F. ROSENE AND A. M. JOEKEL WALTHALL

Introduction

Because of the lack of conclusive data in the literature on the question of whether an individual root hair does actually function as an absorbing mechanism, ROSENE (5) designed an apparatus to measure absorption of water by a single hair. It was used on radish seedlings (*Raphanus sativus*), and the data showed that individual root hairs, originally grown in moist air, absorbed water steadily, with a maximum velocity of 31×10^{-4} mm³/mm²/min and a minimum velocity of 2×10^{-4} mm³/mm²/min when the hairs were almost totally immersed in micropotometers filled with tap water at temperatures ranging from 25° to 31° C. It was also demonstrated that when different regions of an individual root hair were inserted into a micropotometer, there was "unequal absorption in the different areas of the individual cell under the given conditions" (5: 604). The experiments indicated that the distal end of a single hair had a greater velocity of absorption than the relatively more proximal areas.

Up to the present time apparently no other work has been done to determine directly the velocity of absorption of water by root hairs. This paper reports the extension of the procedure used by ROSENE to other species of plants.

Material and methods

The literature contains considerable data upon the growth of root hairs of many plants in different media. These provided a list from which plants were selected for study. In order to use the equipment effectively, it was necessary to select species which would germinate and produce in moist air long root hairs that grew at a suitable rate at right angles to the radicle and were not too close together. Preliminary experiments were performed to determine which species met these conditions. The plants finally selected were: Chinese cabbage (*Brassica chinensis*), Georgia collard (*Brassica oleracea*), mustard (*Brassica hirta*), radish (*Raphanus sativus*), tomato (*Lycopersicon esculentum*), field pea (*Pisum sativum arvense*), corn (*Zea mays*), oat (*Avena sativa*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*).

For a comparison of velocities of water absorption by individual root hairs of

different species ideal conditions should include similar hairs on the various plants. But seedlings of different species vary in rate of growth, in length of the radicle at the same age, and in the distribution and the length of hairs on the radicle. In order, therefore, to have standard conditions, the particular hair selected for insertion into the micropotometer for an experiment was always in a region from 4 to 11 mm. from the apex of the radicle. It was necessary to select hairs from an extended area rather than from a given point because of their stickiness, their proximity to one another, and their occasional overlapping. This, however, complicated the selection of comparable absorbing areas on each hair from one species to another. Such plants as field pea and corn, for example, had very short, stubby hairs over the designated region, while radish and Chinese cabbage had comparatively long, slender hairs. When it was necessary to use longer hairs, the distal end only was inserted in order to study comparable areas. All seedlings were used before the food material in the seed had been exhausted.

In general, the equipment and procedure were those described by ROSENE (5). The essential equipment consisted of a movable holder for the seedling and of movable micropotometers placed within a rectangular glass experimental chamber. The chamber, lined with wet filter paper, fitted into a depression in a rectangular, paraffin-coated transit base which was supported by a heavy rod. Four perforations, fitted with glass guards and sealed with latex rubber finger cots, allowed the root holder, "root position adjuster," window wiper, and thermometer to be inserted through the base. The micropotometer support, with control and experimental micropotometers attached to it, was inserted through a hole in the top of the chamber that was sealed with a finger cot. Distilled water was used to fill the depression and the finger cots and to moisten the filter paper. The micropotometer support and root holder were moved by micromanipulators. The micropotometers were filled with aerated Hoagland's solution at pH *ca.* 5.

A horizontal microscope with a Filar micrometer was used when making adjustments with the micromanipulators and in taking all measurements.

The apparatus was set on a stationary table in a dark basement room. A single fluorescent lamp placed behind the experimental chamber provided a direct light source and was kept on continually during each experiment. Healthy root hairs were produced by the radicle growing in the chamber under the specified conditions. SNOW (7), ROBERTS (4), and JEFFS (3) found the only effect of light on root hairs to be due to its effect on the growth of the plant as a whole.

Tomato, field pea, corn, oat, barley, and wheat seeds were soaked 24 hours before being placed on wet filter paper in Petri dishes for germination in the dark; Chinese cabbage, Georgia collard, mustard, and radish seeds were usually placed dry on wet filter paper in Petri dishes. After radicles appeared, each seedling was placed in the experimental chamber in the evening and used the next morning. Root hairs which had developed and grown entirely inside the moist environment of the chamber were always used in experiments. When a root hair had been selected for study and had been inserted into the experimental micropotometer, the preparation was left 20 minutes for equilibration.

Readings were taken in continuous direct light at 10-minute intervals for 1–2 hours by measuring the change in posi-

tion of the distal menisci in each micropotometer with the Filar micrometer. The diameter and length of the hair inserted were measured in order to determine its surface area for calculation of the velocity of water absorption. Root hairs are not perfect cylinders, owing to their dome-shaped tip, but the small deviation in area from that of a cylinder was considered negligible.

The velocity of absorption of water by an individual hair was calculated by subtracting the volume change of the solution in the control micropotometer from the volume change of the solution in the experimental micropotometer during unit time, and dividing this difference by the surface area of that portion of the hair inserted into the micropotometer. ROSENE (5) found a slight amount of evaporation in the micropotometers in the absence of root hairs and could not eliminate it under these particular conditions.

Rather than make initial and final readings for a prolonged period of absorption by a large number of hairs within each species, it was considered more desirable to follow the time course of absorption and make more measurements on fewer hairs, because this latter procedure provided not only a more detailed picture of water intake by each hair but a useful check on a new quantitative microtechnique.

Results

VELOCITIES OF ABSORPTION WITHIN A SPECIES

The results are summarized in table 1. The number of experiments indicate the number of hairs studied. Measurements were made on a single hair of different radicles except in one instance. Two of the experiments were performed on individual hairs of the same mustard seedling on the same day. The average velocities of absorption by these two root hairs were similar and were the highest average velocities of absorption by individual hairs found within the species. Each average velocity represents the average of all the velocities measured at 10-minute intervals in a single hair; each mean velocity designates the mean of the determinations for all hairs within each species.

The different hairs within a species manifested a wide range in the number of cubic micra of Hoagland's solution which passed through the immersed hair surface in various 10-minute intervals of all the experiments (table 1). No net water intake was found during one or more 10-minute intervals in certain experiments with three species—barley, oats, and wheat. Since no growth was observed while the hairs were inserted into the micropotometers in these experiments, the apparent increase in volume of the micropotometer solution was evidently not due to growth phenomena.

One individual hair of a tomato seedling grew while inserted into the micropotometer for a period of 5 hours. After calculation of the changed absorbing surface owing to the increased length, the average velocity of absorption (0.22 μ^3/μ^2/min) was closely comparable with the absorption velocity (0.24 μ^3/μ^2/min) observed in 10-minute intervals at the beginning of the 5-hour period. This was the only instance of a root hair growing following insertion into the micropotometer, although many species (1) produce root hairs when growing in a liquid medium. FARR (2) and WILSON (8) both found that reversible inhibition of root hair elongation frequently occurred when a seedling was transferred from one medium to another. ROSENE (5) observed

TABLE 1

VELOCITIES OF ABSORPTION (μ^3/μ^2/MIN) OF HOAGLAND'S SOLUTION BY ROOT HAIRS OF DIFFERENT SPECIES
AVERAGE AGE OF SEEDLINGS 60 HOURS. HAIRS IN REGION 4–11 MM. FROM APEX OF RADICLE

Velocities	Barley	Oat	Wheat	Chinese cabbage	Georgia collard	Mustard	Corn	Radish	Tomato	Field pea
	In any one 10-minute interval									
Lowest	−0.72	−0.54	−0.70	0.13	0.05	0.03	0.20	0.88	0.42	0.03
Highest	1.14	2.40	1.41	1.30	1.97	2.90	7.40	4.19	5.20	11.60
	Range and mean velocities for all experiments									
Lowest average	0.07	0.07	0.00	0.61	0.58	0.59	0.46	1.56	2.12	0.85
Mean	0.3	0.5	0.5	0.7	0.7	1.2	1.7	2.0	2.3	4.4
Highest average	0.44	1.92	0.94	0.83	0.93	2.22	4.54	2.50	2.55	7.00
Temperature range °C.	24.2±5.0	23.8±3.0	24.5±3.0	27.7±2.0	23.8±0.7	25.0±2.0	24.5±3.0	29.7±0.6	26.8±0.4	25.4±2.0
No. of experiments	9	9	8	5	6	6	6	4	6	7

reversible inhibition of growth in radish hairs during immersion in micropotometers filled with tap water.

Uniform average velocities of absorption by the root hairs within any one species were not observed. Fairly close agreement was found in the root hairs of tomato and of Chinese cabbage. Several factors, which are considered in another section, appear to account, in part, for the lack of uniformity of average velocities of absorption by different hairs within a species. These include variations in the immersed areas and length (age) of the hairs and the temperature range.

Data from root hairs manifesting the highest average velocity within a species are presented in table 2. Since the highest velocities in oat and corn hairs were observed in experiments of 60-minute duration, averages at 60 minutes were calculated for the other species. Data from oat and corn root hairs showing maximum velocities in experiments of 90-minute duration are included in table 2 for comparison; these are marked with an asterisk.

Fluctuations in unit volume intake per unit area of hair surface per unit time from one 10-minute interval to another in each experiment were found not only in the hairs manifesting the highest velocities of absorption (table 2) but in all hairs within each species. The range of fluctuations in any one experiment, however, was not so great as that found when all the velocities determined at 10-minute intervals in the experiments within a species were compared.

Velocities of absorption by different species

A comparison of the mean velocities of absorption in table 1 shows that the speed of intake through unit areas of hair surface is greatest in field pea and lowest in barley. Chinese cabbage and Georgia collard of the genus *Brassica* had similar mean velocities of absorption, but mustard in the same genus had a somewhat higher velocity. The mean velocity of absorption by radish hairs was higher than those of the other members of Cruciferae studied. The mean velocities of absorption by hairs of oats, barley, and wheat appear to be similar to one another, but corn, also a member of the Gramineae, shows a much higher mean velocity.

The data in table 1 are arranged from left to right in order of magnitude of the mean velocities of absorption. Before any conclusion is made regarding the significance of an apparent rank in the magnitude of velocities of absorption by the root hairs of the different species, it is important to consider the data in relation to immersed areas and temperature.

Velocities of absorption by hairs with different immersed areas

The comparatively lower maximum velocities of the different species in table 2 were, in general, found in root hairs in which the area of immersion was greater in extent. But the experiments in which these maximum velocities were observed were performed at different temperatures. Data from those experiments which were within a relatively small temperature range ($26.6° \pm 0.7°$ C.), however, are arranged in table 3 according to the extent of the immersed areas. The hairs varied in length but had been selected from a region comparatively the same distance from the radicle apex.

With the exception of one hair in Chinese cabbage, the hairs with immersed areas of less than 10,000 μ^2 showed higher average velocities of absorption. The maximum velocity found within Chinese cabbage, however, appeared in this hair,

TABLE 2

DATA FROM ROOT HAIRS MANIFESTING HIGHEST VELOCITIES OF ABSORPTION WITHIN A SPECIES

SPECIES	AGE IN HOURS FROM SEED	LENGTH OF RADICLE FROM SEED TO APEX (MM.)	DISTANCE OF HAIR FROM APEX (MM.)	TOTAL LENGTH OF HAIR (MM.)	LENGTH OF IMMERSED PORTION (MM.)	AREA OF IMMERSED HAIR SURFACE (μ^2)	TEMPERATURE (°C.)	VELOCITIES OF ABSORPTION (μ^3/μ^2/MIN) AT 10-MINUTE INTERVALS FROM ZERO TIME									AVERAGE FOR 60 MINUTES FROM ZERO TIME	AVERAGE FOR 90-MINUTE PERIOD
								10	20	30	40	50	60	70	80	90		
Barley	66	20.5	5.8	0.71	0.41	15,500	24.3	0.57	0.58	0.25	0.35	0.25	0.80	0.25	0.04	0.92	0.47	0.44
Chinese cabbage	54	17.2	5.8	0.76	0.32	9,700	25.9	0.85	0.88	0.74	1.25	0.22	1.15	0.43	1.30	0.65	0.85	0.83
Collard	88	17.2	6.0	1.14	0.40	11,900	24.0	0.91	0.88	0.93	0.88	0.93	0.91	1.09	0.69	1.20	0.90	0.93
Wheat	75	20.4	4.9	1.71	0.47	17,700	26.8±0.1	0.28	0.87	0.76	1.41	0.76	1.36	0.82	1.34	0.88	0.86	0.94
Oats	48	27.4	6.1	0.42	0.35	13,200	20.4±0.2	2.40	2.00	1.75	1.97	1.29	2.13	....	.....	.....	1.92	.....
Mustard	48	9.0	5.2	0.79	0.38	11,500	26.2±0.3	2.22	2.56	1.82	1.90	2.14	1.94	2.34	2.20	2.87	2.09	2.27
Radish	45	20.2	6.3	0.64	0.17	6,300	29.9	1.22	0.27	1.46	3.95	2.36	3.52	2.60	3.00	4.10	2.13	2.50
Tomato	96	22.5	5.8	0.26	0.23	8,700	26.5±0.1	0.42	1.88	4.21	3.49	0.80	5.20	1.36	2.82	2.82	2.66	2.55
Corn	72	30.0	8.0	0.13	0.11	4,000	24.4±0.1	4.97	3.55	4.97	0.37	7.37	6.00	....	.....	.....	4.55	.....
Field pea	49	40.0	10.9	0.17	0.14	7,500	27.3±0.1	4.89	6.57	6.62	7.20	10.53	8.32	6.05	5.88	.....	7.35	7.00
Corn*	72	37.0	9.9	0.24	0.21	7,900	23.2±0.2	1.51	1.72	1.73	2.60	1.87	2.60	3.67	0.86	1.51	2.00	2.00
Oats*	96	50.0	6.3	1.32	0.42	15,800	24.9±0.1	0.25	0.68	0.75	0.51	0.19	0.75	1.12	0.23	0.97	0.52	0.60
Wheat*	41	10.2	3.5	0.37	0.35	13,200	24.1±0.1	1.66	2.26	3.12	3.12	3.14	3.47	5.31	6.28	6.18	2.79	3.91

* Explained in text.

which, within the species, had the smallest immersed area. It was also the shortest Chinese cabbage hair studied, and a greater proportion of its total length was immersed.

The average velocities of absorption observed in five of the seven experiments on field pea hairs appear in table 3. In cubic micra per square micron of surface per minute the seven average velocities were: 0.85, 3.46, 3.57, 4.98, 5.12, 5.73, and 7.0. Only one of the hairs had an immersed area greater than 7900 μ^2. Its immersed area was 14,900 μ^2. It manifested a considerably lower average velocity of absorption, and it was the longest of the hairs, the next in size being 0.28 mm. in length. The shortest hair studied in this species which had a proportionately greater portion inserted had the highest average velocity of absorption.

These data show that at a given temperature it is important to use hairs of the same length and with similar immersed areas in order to compare the mean velocities of absorption of different species. In two experiments with hairs of different species—field pea and corn—the hairs were identical in length (0.13 mm.) and the immersed lengths (0.11 and 0.10 mm., respectively) were practically the same, as were the areas of 4100 and 4000 μ^2, but the average velocities of absorption were different (table 3). This might represent a species difference in absorption velocity.

The problem of studying comparable areas in hairs of the same length in different species is complicated by the variability of hair length in different species. If the longest of the short, stubby hairs of field pea were to be used as a standard of reference, this would mean that comparatively older hairs of field pea would be

TABLE 3

AVERAGE VELOCITIES OF ABSORPTION BY HAIRS WITH DIFFERENT AMOUNTS OF SURFACE IMMERSED AT 26.6° ± 0.7° C.

Species	Temperature (° C.)	Duration of experiment (min.)	Distance of hair from apex of radicle (mm.)	Total length of hair (mm.)	Length of immersed region (mm.)	Area of immersed hair surface (μ^2)	Velocity of absorption μ^3/μ^2/min
Oats	26.4	90	7.1	1.65	0.47	20,700	0.17
Chinese cabbage	26.3	70	6.5	1.27	0.63	18,900	0.61
Chinese cabbage	27.0	70	6.3	1.50	0.62	18,700	0.71
Oats	26.8 ± 0.1	90	4.9	1.71	0.47	17,700	0.23*
Wheat	26.0 ± 0.1	80	5.7	0.72	0.47	17,700	0.31*
Wheat	26.6 ± 0.1	90	6.0	0.51	0.45	17,000	0.94
Field pea	26.4 ± 0.2	90	5.2	0.49	0.34	14,900	0.85
Chinese cabbage	26.7	90	5.5	1.07	0.44	13,300	0.65
Mustard	27.1 ± 0.1	70	4.3	1.05	0.40	12,100	1.82
Mustard	26.2	90	5.2	0.79	0.38	11,500	2.22
Chinese cabbage	25.9	90	5.8	0.76	0.32	9,700	0.83
Tomato	27.0	90	5.8	0.30	0.23	8,700	2.35
Tomato	26.6	90	5.8	0.26	0.23	8,700	2.55
Field pea	27.3	90	5.9	0.21	0.18	7,900	3.46
Field pea	27.3	70	6.1	0.28	0.18	7,900	3.57
Tomato	26.9 ± 0.1	80	6.8	0.32	0.21	7,300	2.26
Field pea	26.7 ± 0.1	60	6.6	0.15	0.12	5,300	5.12
Field pea	26.4	60	5.7	0.13	0.11	4,100	5.73
Corn	27.3	60	8.0	0.13	0.10	4,000	4.54

* Negative values in two 10-minute intervals.

129719

studied, while in radish and wheat, for example, the hairs of this length would be relatively younger. Table 3 shows that a mustard hair 1.05 mm. in length with 38% of the hair immersed (12,100 μ^2) had a lower average velocity of absorption than a shorter (younger) mustard hair, 0.79 mm. in length with 48% of its length immersed (11,500 μ^2).

Effect of temperature upon velocities of absorption of root hairs

Although it was not possible to maintain a day-to-day constant temperature in the basement room used for the experiments, fourteen of the individual experiments showed no change in temperature during 10-minute intervals.

The data of table 2 show that the fluctuations in velocities of absorption which occurred from one 10-minute interval to another during an experiment were independent of temperature change, for they also occurred at constant temperature. Table 2 shows also that the maximum average velocities of absorption were not found in experiments at any one temperature.

The negative values of absorption which appeared during certain 10-minute intervals in the experiments on oats, barley, and wheat were observed at relatively higher temperatures. Further work is needed to establish whether these are due to temperature effects. In another series of experiments on the hairs of wheat only, however, negative values were not found during any 10-minute interval in eighteen experiments performed at a temperature range of 23.9° ± 0.2° C.

The temperatures (average, 23.8° C.) at which absorption by the root hairs of Georgia collard was measured were lower than those (average, 27.7° C.) at which absorption by Chinese cabbage root hairs was measured (table 1). But the mean velocity of absorption for a root hair of Georgia collard was the same as that for a root hair of Chinese cabbage. The areas of immersion in the experiments ranged from 9700 to 18,900 μ^2.

The results in table 4 are from those experiments within a species in which the

TABLE 4

AVERAGE VELOCITIES OF ABSORPTION BY ROOT HAIRS AT DIFFERENT TEMPERATURES

Species	Duration of exp. (min.)	Immersed area	Temperature (° C.)	Velocity of absorption
Field pea	70	4,200	22.6±0.2	4.98
Field pea	60	4,100	26.4	5.73
Corn	90	12,800	21.7±0.3	0.96
Corn	90	11,700	26.0±0.1	0.46
Oats	60	12,400	21.7±0.2	1.7
Oats	90	15,800	24.9±0.1	0.6
Oats	90	17,700	26.2±0.1	0.07*
Oats	90	17,700	26.8±0.6	0.23*
Wheat	90	15,800	21.3±0.1	0.55
Wheat	90	16,200	26.6±0.2	0.24*
Mustard	90	12,000	23.2±0.2	0.91
Mustard	70	12,100	27.1±0.1	1.82

* Negative values during some 10-minute intervals.

areas of immersion were comparable and the temperatures varied *ca.* 4° C. Field pea and mustard hairs showed higher rates of absorption at the higher temperatures, but in oat, wheat, and corn the velocities were lower at the higher temperatures.

Table 2 shows data obtained from two root hairs of wheat with relatively comparable amounts of immersed areas but of different length and from experiments performed at different temperatures. The data designated by an asterisk were from a hair studied in the separate set of experiments on wheat mentioned earlier. The average velocity of absorption in the wheat* hair was over three times greater than that of the wheat hair in table 2, which showed a maximum velocity of absorption in this set of experiments. The mean velocity of absorption in the wheat hairs of the second set of experiments at a temperature of 23.9° ± 0.2° C. was 2.7 μ^3/μ^2/min compared with the mean of 0.5 μ^3 when the range of temperature was 24.5° ± 3.0° (table 1). None of the experiments in the study reported here was designed to study temperature effects, but they do indicate that temperature is important.

Discussion

The direct quantitative measurement of absorption by individual root hairs is a new microtechnique which heretofore had been used but once (**5**); the data then obtained furnished strong evidence for unequal absorption in different areas of an individual root hair. In the present investigation, designed to compare the velocity of absorption in root hairs of different species, the importance of other variables became known as the work progressed.

Evidently the deviations in the average velocities of absorption by root hairs within a species were due, in part, to differences in length, in age, and in the extent of immersed areas of the root hairs as well as differences in temperature. The greatest uniformity in average velocities of absorption within a species was found in tomato. The experiments on tomato were performed within a narrow temperature range (26.8° ± 0.4°C.), the variation in hair length (0.26–0.42 mm.) was not so great as in other experiments, and the immersed areas were fairly similar.

Radish root hairs showed an average velocity of absorption that fell within the range of minimum and maximum velocities reported by ROSENE (**5**) for the same species.

A comparison may be made of the velocities of absorption of the root hairs of corn found in the present study with the results obtained from corn roots by SEELIGER (**6**). A strict comparison is not valid, however, since plants of different age, different temperatures, solutions of different osmotic pressure, and different absorbing regions were studied. She used entire corn plants from 4 to 9 days old, with roots from 44 to 178 mm. long, and with temperatures ranging from 28° C. to 32° C. in a saturated atmosphere. She measured potometrically the velocity of absorption of Hoagland's solution (diluted four times) by five regions of a root simultaneously and found the average absorption velocity of a root to be 0.32 mm^3/mm^2/hr or 5.4 μ^3/μ^2/min. Although this is higher than the average velocity of absorption of corn root hairs (1.7 μ^3/μ^2/min) found in the present study, it is not much higher than the maximum velocity (4.55 μ^3/μ^2/min) observed. SEELIGER did not indicate whether the corn roots she used were hairless; yet a comparison of her data with those of the present investigation raises the question of the relative importance of hair and hairless

epidermal cells with respect to absorption.

At the present stage of our knowledge of the physiology of absorption by individual root hairs it is not possible to attach much significance to the rank in magnitude of absorption velocities shown in tables 1 and 2.

The data showed that the mean velocities of absorption by root hairs of Chinese cabbage, Georgia collard, and mustard of the genus *Brassica* were relatively similar. Yet when the average velocity of a mustard root hair 1.05 mm. long with an immersed area of 12,100 μ^2 is compared with that of a Chinese cabbage root hair 1.05 mm. long and an immersed area of 13,300 μ^2, it shows that absorption by the mustard hair was almost three times as fast as that by the Chinese cabbage hair under comparable conditions of temperature, hair length, and extent of immersed area (table 3).

The fluctuations in the velocity of water absorption by individual root hairs from one 10-minute interval to another, which were independent of temperature changes, were similar to those reported by ROSENE (5). The data in the present investigation indicated no periodicity of absorption by a root hair in any of the species studied.

Individual root hairs were produced, grew, and absorbed water over the range of temperatures used in these experiments. JEFFS (3) reported that a sudden rise or fall of temperature altered the rate of growth of a root hair; if as much as 3° C.–5° C., it stopped growth altogether. The temperature change observed in any one of the present experiments in which a change occurred was gradual. Small gradual changes appeared to have no effect upon the velocity of absorption by the root hairs of any radicle. Oats at temperatures near 26° C. exhibited intermittent, low velocities of absorption by each root hair and at 24° C. exhibited continuous absorption; barley and wheat showed the same behavior to some extent.

Summary

1. ROSENE's micropotometric technique was used to determine the velocity of absorption of Hoagland's solution at a pH of *ca.* 5 by individual root hairs of ten species. Experiments were performed with the seedlings in moist air at different temperatures ranging from 19° C. to 30° C.

2. The mean velocities of absorption by the root hairs within a species calculated in cubic micra per square micron of hair surface per minute were: barley, 0.3; oats, 0.5; wheat, 0.5; Chinese cabbage, 0.7; Georgia collard, 0.7; mustard, 1.2; corn, 1.7; radish, 2.0; tomato, 2.3; and field pea, 4.4.

3. Fluctuations in the velocity of absorption by individual root hairs from one 10-minute interval to another were observed, but no definite periodicity was noted. These fluctuations were independent of temperature changes.

4. Small gradual temperature changes appeared to have no effect on the velocity of absorption by an individual root hair during any one experiment. Oats, barley, and wheat showed intermittent absorption at temperatures above 25° C. and continuous absorption of water at temperatures below 25° C.

5. The results demonstrated that in any study on absorption by individual root hairs the age and length of each hair and the extent of the immersed area, as well as temperature, are important variables.

DEPARTMENT OF ZOÖLOGY
UNIVERSITY OF TEXAS
AUSTIN, TEXAS

LITERATURE CITED

1. FARR, C. H. The formation of root hairs in water. Proc. Iowa Acad. Sci. **32**:157–165. 1925.
2. ———. Studies on the growth of root hairs in solutions. II. The effects of concentrations of calcium nitrate. Amer. Jour. Bot. **14**:497–515. 1927.
3. JEFFS, R. E. The elongation of root hairs as affected by light and temperature. Amer. Jour. Bot. **12**:577–606. 1924.
4. ROBERTS, EDITH A. The epidermal cells of roots. BOT. GAZ. **62**:488–506. 1916.
5. ROSENE, H. F. Quantitative measurement of the velocity of water absorption in individual root hairs by a microtechnique. Plant Physiol. **14**: 588–607. 1943.
6. SEELIGER, LILLIAN N. Studies on the velocity of water absorption in local root regions in relation to transpiration. Thesis. Univ. of Texas, Austin. 1942.
7. SNOW, L. M. The development of root hairs. BOT. GAZ. **40**:12–48. 1904.
8. WILSON, K. The production of root-hairs in relation to the development of the piliferous layer. Ann. Bot. **50**:121–154. 1936.

GROWTH OF THREE VARIETIES OF LILIUM FROM BULBS STORED IN VAPORS OF METHYL ESTER OF NAPHTHALENEACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 608

R. M. ACKER

Introduction

In recent years certain growth-regulating substances have been widely used both to prevent or retard growth of plants or plant parts and to stimulate growth. Relatively high concentrations usually retard growth, while low concentrations may hasten or increase it. Prevention of growth of trees, rose bushes, certain ornamental shrubs, potatoes, carrots, parsnips, turnips, etc., in storage has been demonstrated to be commercially feasible (**3, 5, 6, 7, 8, 10**). Low concentrations of certain growth-regulators have stimulated growth and induced early flowering of rose bushes, snap beans, and pineapple (**7, 9, 12**). No studies on the response of lilies to these compounds are known to the writer.

The purpose of the experiments reported here was to determine the growth responses of three species of lilies to storage in vapors of the methyl ester of naphthaleneacetic acid.

Material and methods

Fifteen hundred bulbs each of Easter lily, var. Estate (*Lilium longiflorum* var. Estate), Easter lily, var. Croft (*L. longiflorum* var. Croft), and Regal lily (*L. regale*) were supplied by the George J. Ball Company of West Chicago, Illinois. The bulbs are hereafter referred to as Estate, Croft, or Regal, respectively.

All bulbs were grown in Oregon. The Estate and Croft lilies were dug in late August and early September, 1947, and held in common storage until October 6, 1947, when they were shipped to West Chicago, Illinois, in a refrigerator car iced at 40° F. In West Chicago they were stored at 32°–34° F. until delivery to the University of Chicago. The Regal lilies were dug in September but had no cold-storage treatment before delivery to the University. The Estate and Croft bulbs were delivered in healthy condition to the University of Chicago greenhouse on November 12, 1947, and the Regal on

November 18, 1947. The bulbs of Estate were 17.8–20.4 cm. in circumference, their moisture content was 65.5%, and the average fresh weight per bulb 64.1 gm. The Croft bulbs were 22.9–25.4 cm. in circumference, their moisture content 62.5%, and average fresh weight per bulb 142.7 gm. The bulbs of Regal lily were 17.8–20.4 cm. in circumference, their moisture content 74.3%, and average fresh weight per bulb 101.4 gm. All bulbs were stored at 34° F. until the experiments were begun.

Methyl ester of naphthaleneacetic acid (MENA) was obtained from the Dow Chemical Company. Lots of 100, 10.0, or 1.0 gm. were dissolved in separate 2-liter amounts of acetone, and each solution was sprayed on a different 5-pound amount of shredded newspaper with a DeVilbiss spray gun at 15 pounds pressure. The paper was continually mixed by hand during the spraying to insure uniform application. One batch sprayed with acetone only was used for packing the control bulbs. All paper was allowed to dry thoroughly before use.

Wooden boxes $10\frac{1}{2}$ inches deep and $11\frac{1}{2}$ inches in inside width and length—a volume of 0.804 cu. ft.—were used for storage. They were lined with heavy waterproof paper to minimize loss of water vapor and volatile growth-regulator. After a $\frac{1}{2}$-inch layer of moist peat moss was placed on the bottom of the box, shredded newspaper from one of the 5-pound amounts was uniformly placed over the peat moss. Sixteen bulbs of Croft lily, twenty-five of Estate, or twenty-five of Regal were then placed in one layer upon the paper. The bulbs were covered with the treated shredded paper, and a $\frac{1}{2}$-inch layer of peat moss was added. This procedure was repeated until the desired number of bulbs was packed. The heavy lining paper was then folded over the bulbs and sealed with cellulose tape.

Fifty bulbs of Croft, one hundred of Estate, or seventy-five of Regal were usually packed in each box. Each filled box contained 0.25 pound of the shredded paper. The bulbs were thus treated at a rate of 0.0, 0.062, 0.62, or 6.2 gm. of MENA per cubic foot of storage space.

After packing, the bulbs were stored at 34° F. or 50° F. until planting time. A cold room supplied with a fan was used for the lower temperature, and incubator ovens for the higher. In all cases the temperature fluctuated only 1 or 2 degrees during the storage period.

After storage for the desired time, the bulbs were planted in 6-inch, unglazed clay pots so that the top of the bulb was about 2 inches below the soil surface. The potting compost was a mixture of two parts of a loam soil to one part leaf mold, with a pH value of 6.9. The pots were placed on a greenhouse bench, and those of each treatment were randomized into three replicate plots. The plants were watered sufficiently to promote normal greenhouse growth. Some bulbs of Regal lily were planted out of doors in rows in a garden at a depth of 6 inches.

Experimentation

Experiment I. Storage of Croft and Estate at 34° F.

The purpose was to determine the effect of storing the bulbs in vapors of MENA upon later growth and development of the plants. On November 16, 1947, 1200 bulbs each of Croft and Estate were packed and then stored at 34° F. One-fourth of the total number of each served as controls, one-fourth was exposed to 0.062 gm. of MENA per cubic foot of storage space, one-fourth to 0.62

gm. per cubic foot, and the remainder to 6.2 gm. per cubic foot. They were examined weekly during storage. On January 9, 1948, after 54 days of treatment, one-third of the bulbs of Estate and Croft of each treatment were planted in pots and placed in the greenhouse. On February 15, after 91 days of treatment, another one-third of the bulbs were planted, and on March 24, after 129 days of storage, the third and last planting was made.

At planting times the moisture percentage of control bulbs was determined, and observations were made frequently during growth. The plants were harvested when most control plants were in full bloom, and data were taken as shown in the tables. At harvest the bulbs and roots were washed off with water to facilitate observations.

The temperature of the greenhouse averaged 60° F. during January, February, and March. During April, May, and the first 3 weeks of June, it seldom exceeded 85° F. The maximum daily temperatures during the laxt week of June and all of July usually exceeded 100° F.

CROFT LILIES, FIRST PLANTING.—At planting time, January 9, the bulbs were turgid and healthy. No differentiation of flower primordia and only slight root development had occurred.

TABLE 1

DATA ON CROFT LILIES GROWN FROM BULBS PREVIOUSLY STORED IN VAPORS OF METHYL ESTER OF NAPHTHALENEACETIC ACID (MENA). VALUES, EXCEPT EMERGENCE, ARE AVERAGES OF PLANTS AT HARVEST

Planting	Storage period in MENA (days)	Gm. of MENA per cu. ft.	Percentage of emergence*†	Height* (cm.)	Dry weight of tops per plant‡ (gm.)	No. of leaves‡	No. of flowers*	No. of bulblets‡	Wt. of bulblets‡
First.....	0	0.0	96.4	21.9	9.4	65.6	3.8	2.1	1.8
	54	0.062	95.1	20.9	9.1	65.6	3.6	2.1	0.8
	54	0.62	85.9	19.4	8.6	62.2	3.3	8.5	3.0
	54	6.2	37.2	17.8	6.0	54.5	2.3	8.8	3.2
Second.....	0	0.0	00.1	29.3	8.2	54.7	2.6	5.4	20.7
	91	0.062	98.7	27.7	11.2	57.2	2.9	3.1	12.1
	91	0.62	44.0	27.5	8.9	54.6	2.4	8.2	39.1
	91	6.2	0.0	20.1	3.9	36.1	1.5	8.0	34.7
Third.....	0	0.0	72.2	34.1	7.4	50.6	2.4	4.1	9.9
	129	0.062	72.4	34.1	8.7	49.9	2.6	3.7	5.2
	129	0.62	80.6	31.3	8.3	50.1	2.3	7.1	16.6
	129	6.2	2.2	19.7	2.6	39.1	1.3	6.7	13.3

* Based on seventy-two plants.

† Emergence counts taken 32, 24, and 27 days after planting for first, second, and third plantings, respectively. Final emergences in all treatments were about 100%.

‡ Based on thirty plants.

Emergence counts made on February 9, 32 days after planting and before all plants had emerged, showed that growth from bulbs previously stored in 6.2 gm. of MENA per cubic foot was much retarded (table 1). The average heights—measured as the distance between the soil surface and the tips of the leaves or stem—of control plants and of those from bulbs stored in 0.062, 0.62, and 6.2 gm. of MENA per cubic foot were 10.2, 7.7, 7.7, and 0.4 cm., respectively. Bulbs from the 6.2-gm. treatment had no visible roots, but those of other treat-

ments and controls had produced them from the lower three nodes of the stems and from the bases of the bulbs. The first three nodes of the stem were below the soil surface.

Final observations and harvesting were accomplished on April 24, 107 days after planting, when most of the control plants were profusely flowering. Plants from bulbs previously stored in 6.2 gm. of the growth-regulator per cubic foot were much stunted and those from the 0.62-gm. treatment slightly so (table 1). These plants produced fewer leaves than the controls.

Flowering occurred earliest in plants grown from bulbs previously stored in 0.062 gm. of MENA per cubic foot (table 2). At harvest time many of these flowers were in anthesis or had already withered. In the other lots treated with MENA over half the flowers were still in the bud stage.

Many bulblets about 0.6 cm. in diameter were found below the soil surface on stems grown from bulbs previously treated with 6.2 or 0.62 gm. of MENA per cubic foot (table 1). The average weight of bulblets on plants from the 0.062-gm. treatment was less than that of the controls.

The root systems of plants from the 0.62-gm. treatment were more extensive than those of any other treatment. Most of these roots arose from the stem and not from the bulb. The root systems of control plants and of plants from the 0.062-gm. treatment were similar to, although less extensive than, those of plants from the 0.62-gm. treatment. Plants from bulbs previously exposed to the highest concentration of MENA showed no roots at the base of the bulb.

CROFT LILIES, SECOND PLANTING.—At planting time, February 15, all bulbs were turgid and apparently healthy. Dis-

TABLE 2

PERCENTAGES OF FLOWERS OF CROFT LILY IN DIFFERENT STAGES OF DEVELOPMENT AT HARVEST TIME, 107, 97, AND 96 DAYS AFTER PLANTING FOR FIRST, SECOND, AND THIRD PLANTINGS, RESPECTIVELY. PLANTS GROWN FROM BULBS STORED IN DIFFERENT CONCENTRATIONS OF METHYL ESTER OF NAPHTHALENEACETIC ACID (MENA). AT LEAST 82 PLANTS PER TREATMENT

Planting	Storage period in MENA (days)	Gm. of MENA per cu. ft.	Stage				
			In bud	Buds opening	Prime	Anthesis	Fading or withered
First.....	0	0.0	35.8	9.8	11.6	34.6	8.2
	54	0.062	20.7	6.2	3.2	45.6	24.3
	54	0.62	60.7	9.9	8.4	16.5	4.5
	54	6.2	68.3	10.8	6.3	14.6	0.0
Second....	0	0.0	70.7	6.7	13.0	7.0	2.6
	91	0.062	16.5	0.2	15.3	36.4	31.6
	91	0.62	38.2	2.2	20.0	33.3	6.3
	91	6.2	38.1	2.2	12.3	36.3	11.1
Third.....	0	0.0	45.6	8.9	11.4	22.8	11.3
	129	0.062	10.6	6.3	4.4	27.6	51.1
	129	0.62	31.7	0.2	17.0	26.8	24.3
	129	6.2	8.3	12.5	4.1	33.3	41.8

section showed that stem elongation had occurred only in the treated bulbs. Stems in bulbs treated with 0.062 or 0.62 gm. of MENA per cubic foot were about 0.5 cm. long, and those in the 6.2-gm. treatment were 2.3 cm. long. The boxes containing the latter bulbs were very moist.

Emergence counts were made on March 9, 24 days after planting but before all plants had emerged (table 1). Percentage emergence and heights showed that 0.062 gm. of MENA per cubic foot had stimulated growth and that 6.2 gm. inhibited it. Control plants and plants from bulbs previously treated with 0.062, 0.62, or 6.2 gm. of MENA per cubic foot were 0.6, 5.1, 1.9, or 0 cm. in height, respectively, when heights of emerged plants only were used.

By April 8 the increased size of plants from the 0.062-gm. treatment was striking (fig. 1*A*). Those from the 0.62-gm. treatment showed some stimulation, but those from the 6.2-gm. treatment were retarded. The average heights of control plants and of plants from bulbs previously treated with 0.062, 0.62, or 6.2 gm. of MENA per cubic foot were 17.7, 22.9, 20.4, or 15.3 cm., respectively.

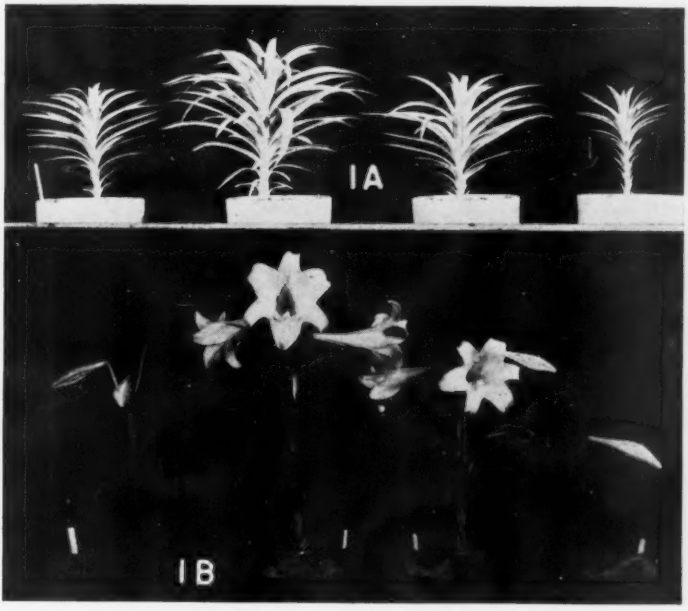


FIG. 1.—(*Left to right*). Croft lilies from bulbs previously stored for 91 days in 0.0, 0.062, 0.62, or 6.2 gm. of MENA per cubic foot. *A*, 59; *B*, 91 days after planting. Note that plants from bulbs treated with 0.062 gm. of MENA per cubic foot are much larger than the others, including controls.

Plants from the 0.062-gm. treatment were taller and bushier, and some flower buds had appeared. Many plants from the 0.62-gm. treatment had formed some flower buds, but bud development in control plants was retarded.

Final observations were made and harvesting done on May 21, 97 days after planting (table 1, fig. 1*B*). Plants from bulbs previously treated with 0.62 gm. of MENA per cubic foot were larger,

but those from the 6.2-gm. treatment were smaller, than the controls. Dry weight of tops and numbers of flowers and leaves per plant were greatest in plants from bulbs treated with the lowest concentration of MENA. Plants from bulbs previously treated with 0.062, 0.62, or 6.2 gm. of MENA per cubic foot flowered 2 weeks earlier than controls (table 2). Plants from bulbs treated with 0.062 or 0.62 gm. of the growth-regulator produced larger flowers than the control plants (table 3, fig. 2). Flowers were measured on overcast days, the time these "stimulated" flowers were the largest. On hot, sunny days the "stimulated" flowers evidently shrunk but did not wilt. Microscopic examination indicated that the epidermal cells of petals of "stimulated" flowers were larger than those of control flowers. Flower size was determined by measuring the length and width of the flowers; the former being the distance between the base of the flower and tip of a petal when the petal was straightened out, the latter the distance between the outside of one sepal to the outside of the opposite petal as measured with calipers. The greatest number and weight of bulblets were produced on plants from the 0.62-gm. or 6.2-gm. treatment (fig. 3*A*).

After the tops were cut off for dry-weight measurements, many of the plants were kept for further observation. By July 1, 40 days after the tops were cut off, at least 40% of the bulblets on

TABLE 3

SIZE OF FLOWERS OF CROFT LILIES FROM BULBS PREVIOUSLY STORED IN DIFFERENT CONCENTRATIONS OF METHYL ESTER OF NAPHTHALENEACETIC ACID (MENA). MEASUREMENTS MADE 97 AND 96 DAYS AFTER PLANTING FROM SECOND AND THIRD PLANTINGS, RESPECTIVELY. EACH FIGURE IS AVERAGE OF SEVENTY-TWO MEASUREMENTS

Planting	Storage period in MENA (days)	Gm. of MENA per cu. ft.	Length (cm.)	Width (cm.)	Length × width
Second....	0	0.0	18.8	13.5	243.8
	91	0.062	19.7	14.6	277.6
	91	0.62	19.5	13.4	261.3
	91	6.2	17.6	12.1	213.0
Third.....	0	0.0	18.2	13.6	247.5
	129	0.062	19.1	14.5	276.9
	129	0.62	18.7	13.5	252.4
	129	6.2	15.1	10.5	158.5

FIG. 2.—Croft lilies. (*Right*) grown from bulb stored in 0.062 gm. of MENA per cubic foot for 91 days. (*Left*) control. Photograph taken 97 days after planting. Note greater size of flower produced from treated bulb.

plants from the 6.2-gm. treatment had produced stems and leaves (fig. 3*B*). Similar sprouting failed to occur in other treatments.

CROFT LILIES, THIRD PLANTING.—At planting time all bulbs except those treated with 6.2 gm. of MENA per cubic foot appeared dormant. The latter usually had stems protruding from their tips, and their outer scales were rotting and many had fallen off. The moisture content of the bulbs in all lots was about the same as at the beginning of storage.

tration of MENA averaged 3.9 cm. long and had dead tips.

Emergence counts were made on April 19, 27 days after planting (table 1). At this time the average heights for controls and for plants from bulbs stored in 0.062, 0.62, and 6.2 gm. of growth-regulator per cubic foot were 10.2, 7.7, 7.7, and 2.6 cm., respectively. The plants were harvested on June 27, 96 days after planting, when the controls were flowering (table 1). The results were similar to those of the second planting, although

FIG. 3.—Bulbs of Croft lily. *A*, 100 days after planting. Before planting, bulbs were stored for 91 days in (*left to right*) 0.0, 0.062, 0.62, and 6.2 gm. of MENA per cubic foot. Note that greatest number and size of bulblets were produced on bulbs previously stored with 0.62 or 6.2 gm. of growth-regulator per cubic foot. *B*, 137 days after planting and 40 days after blooming and cutting off of tops. Bulb stored in 6.2 gm. of MENA per cubic foot. Note sprouting of bulblets.

Dissection showed that the stem axis of controls had not elongated in storage. Stems of bulbs from the 0.062- and 0.62-gm. lots were 0.5 cm. long; those from the 6.2-gm. lot were 3 cm. in length. Control bulbs had formed no roots, but roots had grown from the scale axis of all treated bulbs. There were from nine to fifteen roots 1.3–15.3 cm. long on bulbs treated with the lowest concentration of MENA. Bulbs treated with 0.62 gm. of MENA per cubic foot had roots averaging 3.9 cm. long. These root tips were swollen and sometimes blackened. Roots on bulbs treated with the high concen-

less pronounced. Plants from bulbs treated with the low and medium concentrations of MENA produced greater dry weight of shoots than controls, while those from bulbs treated with the high concentration were much stunted. Plants from the 0.062- and 0.62-gm. lots flowered earlier and were larger than the controls (tables 2, 3). The greatest weight of bulblets produced by the portion of the stem in the soil was found on plants from the 0.62-gm. treatment.

Growth in the third planting was much more irregular than in the second. This irregularity was probably associ-

ated with the prolonged storage period undergone by these bulbs.

ESTATE LILIES, FIRST PLANTING.—At planting time, January 9, all bulbs were dormant. Growth of plants from bulbs stored in 0.62 or 6.2 gm. of growth-regulator per cubic foot was much retarded. For example, on February 9, 32 days after planting, the average heights of controls and of plants from the 0.062-, 0.62-, and 6.2-gm. lots were 10.2, 13.4, 10.0, and 5.1 cm., respectively.

TABLE 4

DATA ON ESTATE LILIES GROWN FROM BULBS PREVIOUSLY STORED IN VAPORS OF METHYL ESTER OF NAPHTHALENEACETIC ACID (MENA). VALUES, EXCEPT EMERGENCE, ARE AVERAGES OF PLANTS AT HARVEST

Planting	Storage period in MENA (days)	Gm. of MENA per cu. ft.	Percentage of emergence*†	Height* (cm.)	Dry weight of tops‡ (gm.)	No. of leaves‡	No. of flowers*
First.....	0	0.0	60.3	25.9	7.4	65.6	2.4
	54	0.062	60.0	24.7	7.2	65.2	2.5
	54	0.62	13.1	32.2	7.2	67.4	2.0
	54	6.2	20.7	25.8	3.4	49.1	1.4
Second....	0	0.0	72.5	37.1	7.8	63.4	2.4
	91	0.062	86.2	40.9	8.1	62.9	2.8
	91	0.62	75.1	36.8	7.6	65.1	2.3
	91	6.2	33.3	22.6	3.0	41.0	0.8
Third.....	0	0.0	75.1	45.7	7.2	59.1	1.9
	129	0.062	79.8	43.7	7.7	58.3	2.2
	129	0.62	71.0	42.0	6.9	60.0	1.6
	129	6.2	30.2	20.8	2.2	37.2	0.6

* Based on seventy-two plants.

† Emergence counts taken 32, 40, and 27 days after planting for first, second, and third plantings, respectively. Final emergences in all treatments were about 100%.

‡ Based on thirty plants.

ESTATE LILIES, SECOND PLANTING.—At the second planting, February 15, the bulbs were turgid, but the stem axes of bulbs of all treatments, including controls, had elongated about 0.2 cm. The moisture content of the bulbs was about the same as at the beginning of storage.

Emergence counts made on March 25, 40 days after planting, demonstrated that the 6.2-gm. treatment retarded growth while the lowest rate slightly stimulated it. Average height of controls and of plants from the 0.062-, 0.62-, and 6.2-gm. lots were 10.2, 13.4, 10.0, and 5.1 cm., respectively.

The plants were harvested on May 21, 134 days after planting. Those from bulbs previously treated with the high concentration of MENA were stunted, and no significant stimulation was apparent in any treatment (table 4).

Although the average height of control plants was 20.4 cm. by April 19, their flower buds were not visible. The average height of plants from the 0.062-gm. treatment was 25.4 cm., and many flower buds had appeared. Some buds were 1.9 cm. long. Plants from bulbs previously treated with the intermediate concentration of growth-regulator were about the same size as the controls, but those from the 6.2-gm. lot were only 15.3 cm. tall and had no visible flower buds.

Final observations were made on June

18, 125 days after planting (table 4). Plants from the 0.062-gm. treatment were the tallest, heaviest, and produced the most flowers per plant. Plants from the 0.62-gm. lot were the same size as controls, but those treated with 6.2 gm. were much stunted. Plants from bulbs treated with the low, medium, and high concentrations of MENA bloomed 10 days to 2 weeks before the control plants (table 5).

Growth of plants receiving the same treatment was very irregular. This probably resulted from the small size of the bulbs, the long storage period, and the high temperatures prevailing during the latter part of June. Even under the suitable temperatures for growth of the first and second plantings, development was often irregular.

The plants were harvested on July 1, 100 days after planting (table 4). In general, plants of all treatments appeared similar, except that those from the 6.2-gm. treatment were much stunted. Plants from all treated bulbs flowered earlier than controls (table 5).

TABLE 5

PERCENTAGE OF FLOWERS OF ESTATE LILY IN DIFFERENT STAGES OF DEVELOPMENT AT HARVEST TIME, 134, 125, AND 100 DAYS AFTER PLANTING FOR FIRST, SECOND, AND THIRD PLANTINGS, RESPECTIVELY. PLANTS GROWN FROM BULBS STORED IN DIFFERENT CONCENTRATIONS OF METHYL ESTER OF NAPHTHALENEACETIC ACID (MENA)

Planting	Storage period in MENA (days)	Gm. of MENA per cu. ft.	Stage				
			In bud	Buds opening	Prime	Anthesis	Fading or withered
First.....	0	0.0	48.4	6.2	6.1	21.1	18.2
	54	0.062	30.5	4.9	2.9	25.9	35.8
	54	0.62	41.4	6.1	2.3	19.5	30.7
	54	6.2	52.7	11.5	3.8	6.6	25.4
Second....	0	0.0	40.6	7.3	18.0	26.9	7.2
	91	0.062	16.3	2.0	16.1	35.2	30.4
	91	0.62	18.0	3.1	15.5	36.7	28.7
	91	6.2	14.8	3.5	31.0	21.8	28.9
Third.....	0	0.0	41.7	7.5	17.3	25.7	7.8
	129	0.062	15.6	2.1	15.9	34.5	31.9
	129	0.62	16.2	2.1	13.2	42.3	26.2
	129	6.2	11.1	2.3	26.1	36.6	23.9

ESTATE LILIES, THIRD PLANTING.—When the turgid bulbs were planted on March 24, the stem axes of control plants had not elongated. The stem axes of bulbs stored in 0.062, 0.62, and 6.2 gm. of MENA per cubic foot were 0.4, 3.0, and 3.3 cm. in length, respectively.

Emergence counts were made on April 19, 27 days after planting (table 4). At this time the average height of plants from the 6.2-gm. treatment was only 1.3 cm., but plants from the 0.062- and 0.62-gm. treatments had the same height as controls—about 7.7 cm.

EXPERIMENT II. REMOVAL OF REGAL BULBS FROM VAPORS OF MENA AT VARIOUS INTERVALS BEFORE PLANTING

The purpose was to study the effects of exposure of bulbs of Regal to MENA for different lengths of time upon the subsequent growth of the plants. On November 19, 900 bulbs were packed

into four groups of boxes to be exposed to 0.0, 0.062, 0.62, or 6.2 gm. of MENA per cubic foot at 34° F. On March 5, 1948, one-third of the bulbs of each treatment were repacked. The bulbs were removed from the boxes containing shredded newspaper, packed with vermiculite in other boxes, and then returned to cold storage. Thus, there was no MENA vapor in the newly packed boxes except that amount which the bulbs had absorbed during their previous storage. A second one-third lot from each treatment was similarly repacked on April 15, and on April 22 all bulbs were planted in the garden. The three lots had been removed from the vapors of MENA 49 days, 8 days, or 0 days before planting and, therefore, had been exposed to the growth-regulator for 108, 149, or 156 days.

The controls and bulbs treated with 0.062 or 0.62 gm. of MENA per cubic foot were dormant and firm when repacked on March 5. The outer scales of many bulbs exposed to the highest concentration of growth-regulator were rotting and readily fell off. No bulbs had leaves protruding from their tips.

Five-sixths of the control bulbs repacked on April 15 were still dormant, but the remainder of the controls had leaves 0.15–0.6 cm. in length protruding from their tips. About one-fourth of the bulbs stored in the 0.062- or 0.62-gm. concentrations had leaves 0.6–3.9 cm. in length protruding from their tips. Bulbs stored in the highest concentration of growth-regulator were shriveled, and the outer bulb scales were dried up even though nearly all these bulbs had leaves which protruded from 1.3 to 7.7 cm. These shoots showed some curvature and crinkling of leaves caused by the growth-regulator.

Observations on growth were made as in experiment I. The temperature was relatively low during April and May but

TABLE 6

DATA ON REGAL LILIES GROWN FROM BULBS REMOVED FROM METHYL ESTER OF NAPHTHALENEACETIC ACID (MENA) VAPORS AT DIFFERENT LENGTHS OF TIME BEFORE PLANTING IN GARDEN

Storage time in MENA (days)	Length of time bulbs removed from MENA before planting	Gm. of MENA per cu. ft.	Percentage emergence 12 days after planting	Height* (cm.)	Fresh weight* (gm.)	No. of flowers*
.....	.....	0.0	86.1	53.4	35.4	2.0
108.....	49	0.062	93.3	57.9	41.5	2.2
108.....	49	0.62	84.7	60.2	36.6	2.1
108.....	49	6.2	25.0	37.4	26.3	1.1
.....	.....	0.0	87.2	53.6	34.7	2.5
149.....	8	0.062	86.9	54.6	34.4	2.0
149.....	8	0.62	85.8	56.7	38.8	2.0
149.....	8	6.2	18.5	30.8	16.2	1.0
.....	.....	0.0	85.9	52.6	34.2	2.2
156.....	1	0.062	88.3	53.9	40.4	2.5
156.....	1	0.62	89.6	57.4	42.4	2.9
156.....	1	6.2	16.8	30.5	15.3	0.9

* Based on sixty plants.

during the latter part of June was usually high.

Emergence counts of plants from bulbs repacked on March 5 and planted on April 22 were made 12 days after planting (table 6). At this time the average heights of plants from the 6.2-gm. treatment was 8.3 cm.; plants of control and other treatments averaged 25.4 cm. Final observations on July 10 showed that plants from the 6.2-gm. treatment were much stunted (table 6). Their root systems were greatly thickened; the individual roots were often forty times as thick as those of controls (fig. 4). Roots of plants from the 0.62-gm. treatment were sometimes three times as thick as those of controls.

Plants from bulbs stored in MENA for 156 days grew similarly to those stored in the growth-regulator for 149 days. These plants from the 6.2-gm. treatment were stunted. The retardation of growth from the latter bulbs was greater than that from bulbs similarly treated but stored in MENA for only 108 days.

Experiment III. Storage of Regal, Croft, and Estate at 50° F.

The purpose was to study the effects of exposing bulbs to vapors of MENA at 50° F. upon subsequent development of bulbs and growth of plants. One objective was to inhibit sprouting of bulbs at 50° F. When bulbs are stored at room temperature or even in common storage, the stems often grow. Bulbs which have sprouted do not grow vigorously when planted.

On November 18, 1947, four wooden boxes were filled with bulbs of Croft, four with Estate, and four with Regal. Each box contained fifty bulbs of Estate or Croft, or seventy-five of Regal. Boxes contained 0.0, 0.062, 0.62, or 6.2 gm. of MENA per cubic foot, and each treatment consisted of one box of bulbs.

After packing, the boxes were immediately placed in the 34° F. cold-storage room, and on January 5 all boxes were moved to the 50° F. chambers.

Regal lilies.—On February 3, 30 days after the bulbs were moved to the 50° chambers, dissection showed that the stem axes in all treatments were from 2 to 2.5 cm. in length.

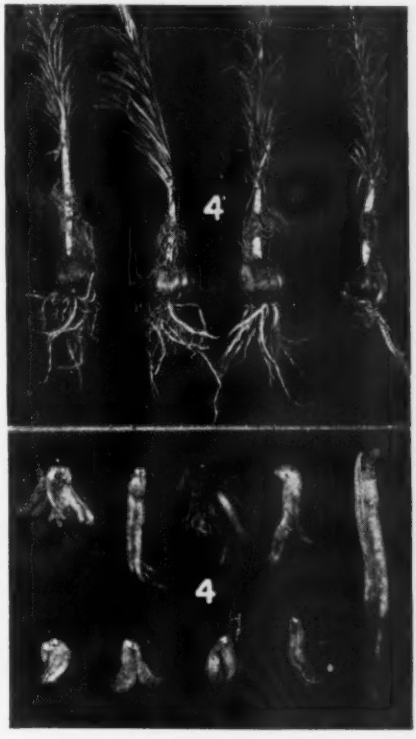


Fig. 4.—Regal lilies. (*Upper*) grown from bulbs previously stored for 149 days in (*left to right*) 0.0, 0.062, 0.62, and 6.2 gm. of MENA per cubic foot. Note that plants from bulbs previously treated with lowest concentration of MENA have the most extensive root systems and that plants treated with highest concentrations have greatly thickened roots. (*Lower*) close-up of detached roots of plants from 6.2-gm. treatment. Photographs taken 81 days after planting.

The bulbs were planted in soil in pots in the greenhouse on March 3, after 59 days of storage at 50° F. Some bulbs in all treatments sprouted so that leaf and stem tips protruded from 5.1 to 7.7 cm. from the tips of the bulbs (figs. 5*A*, 6). The control bulbs and those from the 0.062-gm. treatment were turgid and had produced several roots from 2.6 to 10.2 cm. long. Roots from bulbs treated with the intermediate concentration of MENA were usually only 1.3 cm. long but were two or three times as thick as those of controls. Bulbs stored in the highest concentration of growth-regulator were soft, and many of their shoots were beginning to rot, especially in a 2.6-cm. segment of stem just above the bulb. There were many round excrescences from 1 to 3 cm. in length on the lower portion of the stems. The stems were yellowish and usually one and a half times as thick as stems of the controls (fig. 5*A*). Some stem tips were rotting. The leaf tips of the plants treated with the highest concentration were crinkly, wavy, and often rolled inward. These leaves were only half as broad as those of the controls. Rooting was inhibited, although

FIG. 5.—Bulbs of Regal lily after 48 days of storage at 34° F. and 59 days of storage at 50° F. *A*, upper row of bulbs are controls and lower have been treated at 6.2 gm. of MENA per cubic foot. Note root excrescences, narrow and rolled leaves, and stem curvature in plants from treated bulbs. *B*, 29 days after planting from bulbs stored in (*left to right*) 0.0, 0.062, 0.62, and 6.2 gm. of MENA per cubic foot. Note that increased root production in plants previously treated with growth-regulator is especially evident in 6.2-gm. treatment.

FIG. 6.—Bulb of Regal lily after 48 days of storage at 34° F. and 59 days of storage at 50° F. in 6.2 gm. of MENA per cubic foot. Note root primordia, narrow leaves, and lack of roots from base of bulb.

some bulbs treated with the highest concentration of growth-regulator had two to four roots protruding about 1.3 cm. The inside of the boxes containing 6.2 gm. of MENA per cubic foot was moist.

On April 8, plants from bulbs previously treated with 6.2 gm. of growth-regulator per cubic foot had an average height of 33.1 cm. Average height of control plants and of those of other treatments was 43.2 cm. Controls were rooting both from the base of the bulb and from the lower portion of the stem above the bulb (fig. 5*B*). Plants from bulbs treated with the lowest concentration of growth-regulator produced more roots than controls. Many roots growing from the base of the bulbs from the 0.62-gm. treatment were greatly thickened; no roots grew from the base of bulbs from the 6.2-gm. treatment, but rooting from the stem portion immediately above the tip of the bulbs was greatly stimulated.

Final observations were made on June 18, 108 days after planting, when controls were 61.1 cm. in height and flowering. Plants from the 0.062- and 0.62-gm. treatments were similar to controls, but those from the 6.2-gm. treatment were severely stunted, being only 38.6 cm. high. About 70% of these latter plants failed to flower. Fresh weights of tops per plant for plants previously treated with 0.0, 0.062, 0.62, or 6.2 gm. of growth-regulator per cubic foot averaged 47.2, 56.1, 49.4, or 28.4 gm., respectively.

CROFT LILIES.—The Croft lilies were planted on March 24, when the stems in all treatments protruded above the tips of the bulbs. Final observations were made on July 2, 101 days after planting, when the average heights for plants previously treated with 0.0, 0.062, 0.62, or 6.2 gm. of MENA per cubic foot were 38.4, 38.1, 37.4, or 17.2 cm., respectively. In no instance had stimulation occurred.

ESTATE LILIES.—The Estate lilies were planted on March 24, when the tips of the stems were just emerging from the bulbs. Final observations were made on July 12, 111 days after planting. Growth of plants was similar to that of Croft and Regal. The highest concentration of MENA retarded growth, but the two lower concentrations had little or no effect on over-all growth.

Discussion

Croft and Estate lilies from bulbs previously stored in 0.062 or 0.62 gm. of MENA per cubic foot of storage space at a temperature of 34° F. consistently emerged from the soil earlier and came into bloom about 2 weeks before the controls. This is in agreement with results of several other investigators using various plants. SWARTLEY and CHADWICK (**11**) treated seeds with naphthaleneacetic acid and certain other growth-regulators and reported an increase in percentage of germination. They also showed that treatment of seeds of *Chrysanthemum coccineum* and *Dianthus allwoodii* resulted in significant increases in subsequent growth. It was pointed out by MARTH (**7**) that concentrations of MENA less than 0.1 gm. per 1000 cubic feet resulted in acceleration of sprouting of rose bushes. ALLARD *et al.* (**1**) found that germination and seedling development of species such as sorghum, peas, and navy beans appeared to be stimulated when treated with low concentrations of growth-regulators.

VAN OVERBEEK (**12**) was able to induce flowering in Cabezona pineapple plants by treating the crown of the plant with naphthaleneacetic acid. CLARK and KERNS (**2**) pointed out that aqueous solutions of naphthaleneacetic acid sprayed on the foliage of pineapple plants resulted in flowering 2 months earlier than con-

trols, and MURNEEK *et al.* (9) reported that snap beans matured 5 or 6 days earlier as a result of spraying with naphthaleneacetamide.

In the experiment reported here relatively high concentrations of the growth-regulator were used. The amount of compound which actually came in contact with the sensitive growing point may have been very small, since the latter was tightly inclosed by the fleshy scales of the bulbs. The concentration of the growth-regulator in the air was unknown. Perhaps the compound diffused very slowly from the shredded paper, so that there was only a low concentration present at any given time. The use of airtight containers containing only vapors of the growth-regulator would afford more accurate information regarding the actual concentration present.

In the experiments with the Regal lily the highest concentration of MENA stimulated sprouting during storage more than any other treatment. When the leaves and stems protruded and were no longer protected by the bulb scales, they were severely injured and the subsequent growth greatly inhibited. Bulbs of Croft and Estate lilies stored in the two lower concentrations of MENA usually produced plants that were much larger than controls. This greater size was particularly striking during the earlier growth stages, but it often persisted through flowering. In some cases, however, the control plants appeared to be as large as the "stimulated" plants at time of flowering. In all experiments a pronounced effect of MENA was the stimulation of root development. All lilies used in this experiment produce many adventitious roots from the stems which perform the normal functions of uptake of water and mineral nutrients. Since a much more extensive root system was often formed by the treated plants, it is possible that this accounts in part for the larger plants and flowers produced in some of the treatments. The effects of MENA on the growth of a plant such as tulip would be of great interest, for here the early development of an extensive root system is necessary for the production of a satisfactory crop.

Since it is widely known that growth-regulators affect the carbohydrate metabolism of plants, it is possible that MENA may have affected the carbohydrate metabolism of the bulb in such a manner that stimulation of the young growing shoots resulted. This would account for earlier emergence of shoots through the soil and also the subsequent earliness of flowering.

After control plants of Croft lilies were harvested, the bulbs apparently went into a rest period, because no new stems appeared. Bulbs from plants which had originally been treated with the highest concentration of MENA produced many bulblets which began to sprout soon after harvest time. The growth-regulator evidently prevented or broke the normal rest period.

Summary

1. Bulbs of Easter lily, var. Estate, Easter lily, var. Croft, and Regal lily were packed in wooden boxes with shredded newspaper previously treated with the methyl ester of naphthaleneacetic acid (MENA) to provide concentrations of 0.0, 0.062, 0.62, or 6.2 gm. of growth-regulator per cubic foot of storage space. The boxes were then stored at 34° F. or, in some cases, later transferred to 50° F. Observations were made during storage and later after the bulbs were planted in the greenhouse or garden. At flowering time dry weight of tops, height, blooming time, size and number of flowers per

plant, and weight of bulblets were usually obtained.

2. In one experiment Croft and Estate bulbs were stored at 34° F. One-third of each type of bulb was planted in the greenhouse 54 days later; another one-third after 91 days; and the remainder 129 days after storing. Plants from bulbs treated with the two lower concentrations of MENA flowered 2 weeks earlier than controls at all plantings. Plants from bulbs treated with 0.062 or 0.62 gm. of MENA per cubic foot in the second and third plantings produced greater dry weight of shoots and larger flowers than the controls. In all Croft plantings a greater number of bulblets was produced on the plants previously treated with 0.62 or 6.2 gm. of the growth-regulator.

3. In a second experiment, Regal bulbs were stored in MENA at 34° F. for 108, 149, or 156 days, but they were all planted at the same time in the garden. Plants of all treatments grew at about the same rate, except that those from bulbs previously treated with the highest rate of MENA were stunted. The longer period of time bulbs were exposed to the highest rate of growth-regulator, the greater was the stunting. The two lower treatments of MENA greatly stimulated root production. The highest rate of MENA resulted in the production of many roots, but these were fasciated and greatly thickened and tumor-like.

4. In a third experiment, Estate, Croft, and Regal lily bulbs were stored in MENA for 48 days at 34° F. and for 59 days at 50° F. Bulbs of all treatments sprouted within 30 days after transfer to the higher temperature. The growth of all plants was about the same, except that those which received the highest concentration of growth-regulator were severely stunted.

The writer wishes to thank Drs. E. J. KRAUS, J. M. BEAL, and R. J. WEAVER for their interest, suggestions, and guidance given during the course of the investigation.

CAMP DETRICK
FREDERICK, MARYLAND

LITERATURE CITED

1. ALLARD, R. W.; DEROSE, H. R.; and SWANSON, C. P. Some effects of plant growth-regulators on seed germination and seedling development. BOT. GAZ. **107**:575–583. 1946.
2. CLARK, H. E., and KERNS, K. R. Control of flowering with phytohormones. Science **95**:536–537. 1942.
3. DENNY, F. E. The use of methyl ester of α-naphthaleneacetic acid for inhibiting sprouting of potato tubers, and an estimate of the amount of chemical retained by tubers. Contrib. Boyce Thompson Inst. **12**:387–403. 1942.
4. GARDNER, F. E.; MARTH, P. C.; and BATJER, L. P. Spraying with plant growth substances for control of pre-harvest drop of apples. Proc. Amer. Soc. Hort. Sci. **37**:415–428. 1940.
5. GUTHRIE, J. D. Inhibition of the growth of buds of potato tubers with the vapor of methyl ester of naphthaleneacetic acid. Contrib. Boyce Thompson Inst. **10**:325–328. 1939.
6. HITCHCOCK, A. E., and ZIMMERMAN, P. W. Summer sprays with potassium α-naphthaleneacetate retard opening of buds on fruit trees. Proc. Amer. Soc. Hort. Sci. **42**:141–145. 1943.
7. MARTH, P. C. Effect of growth-regulating substances on shoot development of roses during common storage. BOT. GAZ. **104**:26–49. 1942.
8. MITCHELL, J. W., and CULLINAN, F. P. Effects of growth-regulating chemicals on the opening of the vegetative and floral buds of peach and pear. Plant Physiol. **17**:16–26. 1942.
9. MURNEEK, A. E.; WITTWER, S. H.; and HEMPHILL, D. D. "Hormone" sprays for snap beans. Proc. Amer. Soc. Hort. Sci. **44**:428–432. 1944.
10. SWARBRICK, T. The effect of various concentrations of naphthoxyacetic acid and naphthaleneacetic acid in inhibiting shoot development in apple, swede, carrot, and potato. Amer. Rept. Agr. Hort. Research, Hort. Abs. **14**:1533. 1944.
11. SWARTLEY, J. C., and CHADWICK, L. C. Effects of synthetic growth substances on cuttings, seeds, and transplants. Ohio Agr. Exp. Sta. Bimonth. Bull. **27** (217):125–144. 1942.
12. VAN OVERBEEK, J. Control of flower formation and fruit size in the pineapple. BOT. GAZ. **108**: 64–73. 1946.

A FURTHER STUDY ON THE NONUTILIZATION OF RUBBER AS A FOOD RESERVE BY GUAYULE

H. M. BENEDICT[1]

Introduction

TRAUB (9), in investigating the utilization of rubber hydrocarbon as a food reserve (as reported by SPENCE and MCCALLUM[8]), showed that even under conditions of severe carbohydrate starvation there was no decrease in weight of rubber per plant and therefore concluded that rubber did not serve as a food reserve for the guayule plant (*Parthenium argentatum* A. Gray).

In various reports of the Emergency Rubber Project (5, 7) and the Special Guayule Research Project data have shown small reductions, bordering on significance, in the weight of rubber per plant. These reported losses usually occurred during that season of the year, February through June, when guayule plants were growing very actively following the winter dormant period. The losses reported by SPENCE and MCCALLUM (8) also occurred during May.

Recent experiments (1) have shown that, even in the greenhouse, results obtained by a certain treatment at one time of the year may not be obtained at another season. For instance, plants grown under conditions of high moisture stress during November to March showed no increase in rubber percentage over those grown in low moisture stress, yet from March to November high moisture stress had resulted in an increase in rubber percentage. It also has been shown (2) that under greenhouse conditions guayule plants grow much more rapidly in the spring and summer months than in the fall and winter months. TRAUB (9) has pointed out the importance of determining whether rubber can serve as a food reserve. Because his carefully controlled and detailed experiments were carried out only during the fall and winter months, it seemed desirable to repeat the experiments during the spring months, with the idea that a greater demand for food reserves might be produced at that season and that some utilization of the rubber hydrocarbon might occur.

[1] Formerly physiologist, U.S. Natural Rubber Research Station, U.S.D.A., Salinas, California.

Material and methods

Seed were sown on February 7, 1949. The plants were grown under optimum moisture, temperature, and nutrient conditions until September, 1947, and were then hardened off under high soil moisture stress for about 4 months. On January 20, 1948, these plants were dug, and 500 of a total of 2500 were selected for uniformity of height, root length, and stem diameter. The tops were cut back to 4 inches above the ground line, and all the leaves and shoots were removed. This reduced the stem to a single stalk 4 inches long and made it easier later to distinguish new stem growth from the original. Care was taken to wash the soil away from the roots at the time of digging to minimize breakage.

One hundred of these plants were selected at random and placed in replicates of ten plants each, and their dry weights,

carbohydrates, resin, and rubber content of the stems and roots were determined. Thus, an accurate idea was obtained as to the condition of the plants at the start of the experiment.

The remaining 400 plants were planted in gravel in ½-gallon crocks, watered with Hoagland's (4) complete nutrient solution, and grown until June 1, 1948. Half of them were allowed to develop normally, but the other half were kept continually defoliated in order to prevent as completely as possible the manufacture of any new food. In defoliating, the new shoots were allowed to elongate, but the developing leaves were removed as soon as their early period of rapid expansion was over or when they were about 1½ inches long. The dry weight of the removed leaves was always determined.

By the harvest date some plants had failed to grow at all; others had sent out shoots considerably below the top of the original stem, and this stem part above the new shoots was dry and dead; still others had put out new roots only very close to the crown, and the old root portion below the new roots was dead and often rotten. Because this death of some of the original tissues during the growth period might lead to false conclusions, it was necessary to select plants at the end of the experiment which could be compared with those at its start. All the plants in which some of the original root tissue had died were discarded. The remainder of the defoliated plants were separated into two groups: (*a*) those in which the new shoots arose within ⅛ inch of the top of the original stem and (*b*) those in which the new shoots started lower down (never more than an inch) on the stem, leaving a dead stub above. Of the undefoliated plants only those in which the new shoot arose within ⅛ inch of the top of the original stem were saved. After this selection fifty plants of each type were available for analysis. These were placed at random into ten replications of five plants each.

In preparation for analysis the plants were separated into leaves, new stems developed during the period of the experiment, the original stems, and the roots. Great care was taken to gather all the roots from the gravel in which the plants had been growing. No attempt was made to separate the new roots from the original roots in the defoliated plants because of the very small number of new roots formed. This root separation was made, however, on the plants that were allowed to develop normally.

Dry weights were determined by drying the plants to constant weight at 65° C. in a ventilated dryer. Rubber and resin contents were determined by the method described by TRAUB (10). The carbohydrates, consisting of free sugars, levulins, inulin, and pentosans, were determined by the methods of TRAUB and SLATTERY (11) and MCRARY and SLATTERY (6). Statistical comparison was made by the analysis of variance of FISHER as described in HAYES and IMMER (3).

Results

The data are presented under four treatments as follows:

1. Original plants, stems or roots.
2. Plants kept defoliated from January 20 to June 1, and in which new shoots emerged within ⅛ inch of the top of the original stem.
3. Same as 2 except that the new shoots started lower down on the original stem.
4. Plants allowed to develop without defoliating from January 20 to June 1.

Repeated defoliation throughout the experiment reduced the dry weight of the original stems by 41% and of the original roots by 38%. When the plants were not defoliated, the stems increased

in dry weight by 74% and the roots by 254%. At the close of the experiment the total dry weight of nondefoliated plants was about seven times that of the original plants and about six and one-half times that of the defoliated plants of treatment 2. The dry weights of the roots of the plants in the two defoliation treatments did not differ significantly from each other, but the plants of treatment 2 produced more new stem and leaf growth than did those of treatment 3, and at the same time their original stems underwent a significantly greater reduction in dry weight (table 1).

The percentage contents of the various compounds in the original stems and roots at the beginning and end of the ex-

TABLE 1

DRY WEIGHTS (GM. PER PLANT) OF GUAYULE PLANTS BEFORE AND AFTER BEING GROWN IN FOLIATED OR DEFOLIATED CONDITION

Treatment	New leaves and stems	Original stems	Original roots	New roots
1. Original plants	.....	0.713	0.671	.....
2. Grown defoliated*	0.715	0.420	0.415	.....
3. Grown defoliated*	0.665	0.485	0.450	.....
4. Grown foliated	4.282	1.240	2.374	1.802
Least sig. diff. $P<0.05$	0.043	0.050	0.045	.....

* Treatments 2 and 3 were identical except that in treatment 2 the new shoots originated within $\frac{1}{8}$ inch of top of stem, while in treatment 3 they originated lower down.

TABLE 2

PERCENTAGE CONTENT OF VARIOUS COMPOUNDS OF GUAYULE PLANTS BEFORE AND AFTER BEING GROWN IN DEFOLIATED OR FOLIATED CONDITION

Treatment no.	Percentage content of					
	Resins	Rubber	Sugars	Levulins	Inulins	Pentosans
	Original stems					
1. Original plants	4.54	4.53	0.48	17.72±.43	1.40±.17	15.39
2. Grown defoliated*	3.06	8.07	.29	0.24±.05	0.05±.01	18.33
3. Grown defoliated*	3.24	7.65	.27	0.28±.04	0.04±.01	18.33
4. Grown foliated	3.82	6.27	.50	5.27±.40	0.20±.05	18.02
Least sig. diff. $P<0.05$	0.36	1.29	0.07	†	†	0.72
	Original Roots					
1. Original plants	5.18	5.00	0.34	12.42±.34	1.39±.16	14.86
2. Grown defoliated*	2.34	8.00	.24	0.18±.03	0.05±.01	13.70
3. Grown defoliated*	2.42	7.89	.25	0.14±.03	0.10±.02	14.09
4. Grown foliated	4.06	5.67	.62	5.07±.32	0.33±.03	14.04
Least sig. diff. $P<0.05$	0.31	1.30	0.06	†	†	0.62

* For difference between treatments 2 and 3 see footnote, table 1.

† Not determinable as variances were not homogeneous. Plus and minus values represent standard error of the mean.

periment are shown in table 2. The important features of the experiment, however, have to do with the weights of the various compounds rather than with their percentages in the defoliated plants at the beginning and end of the experiment. The weights have been plotted for the carbohydrates in figure 1 and for the dry weights, resin, and rubber content in figure 2.

The sugars, which are present only in low amounts in any case, were reduced slightly by defoliating the plants. The levulins and inulin, which make up the principal reserve carbohydrates, were reduced in weight by 99% and 98%, respectively, in both the original roots and the tops in the defoliated plants. On the same bases 30% of the pentosans were lost from the tops and 43% from the roots of the defoliated plants of treatment 2, but not quite so much from the tops and roots of treatment 3.

The original stems and roots lost 60% and 72%, respectively, of their resin when kept defoliated. The loss in the stems in treatment 3 was slightly but significantly less than in treatment 2. No loss in rubber per plant occurred in either the original roots or the stems of any of the defoliated plants, nor was there any significant increase.

For plants that were not repeatedly defoliated, it can be calculated from the data in tables 1 and 2 that the weight per plant of each of the determined constituents increased markedly except the inulin in the roots and stems and the levulins in the stems. The rubber increased from 65.9 to 212.4 mg. per plant in the original stems and roots in this treatment.

Discussion

In this experiment the original stems and roots lost as much as 41 and 38% of their dry weights on being repeatedly defoliated as compared with 25% and 18%, respectively, in the experiment of TRAUB (9); but, in spite of the greater exhaustion of reserves, no loss of rubber on a grams-per-plant basis occurred. These results thus support the conclusion that the rubber hydrocarbon does not serve the guayule plant as a food reserve. They also indicate that the small losses of rubber reported by others in the spring of the year are the results of sampling errors or perhaps of some systematic error that occurs when the plants are harvested during that time of the year. For example, it was suggested that, because of the much wetter and softer soil at the beginning of the period of rapid growth, more of the roots of higher rubber content were harvested than at its end; and, as only the total weights of the plants were determined by these workers, this loss of root material from the last sampling was hidden by the over-all growth of the tops. Thus, the two groups of plants could not be compared validly to show changes in weights of rubber.

The almost complete exhaustion of the levulins and inulin, coupled with that of the other constituents determined, accounts for approximately 70% of the loss in dry weight of the original stems and roots after defoliation. The pentosans determined included both the water-soluble and water-insoluble types; this accounts for the differences in the percentages and total amounts present in these plants and in those of TRAUB (9). Determinations of the hemicellulose and cellulose content might have accounted for the remaining 30% of the loss in the dry weight, but there was not sufficient plant material for these analyses.

The results suggest that the resins may serve as a food reserve, for as much as 75% on a gram-per-plant basis was lost after defoliation. It should be point-

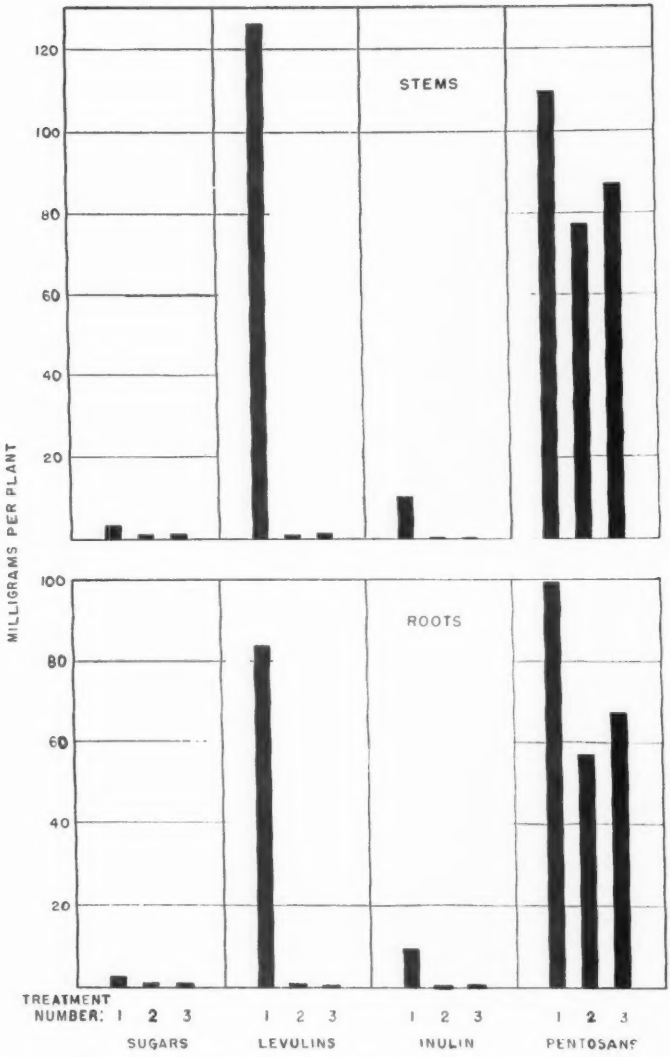


FIG. 1.—Weight of carbohydrates in original stems and roots of guayule plants at the start of experiment (treatment 1) and after being grown with repeated defoliation for some months (treatments 2 and 3).

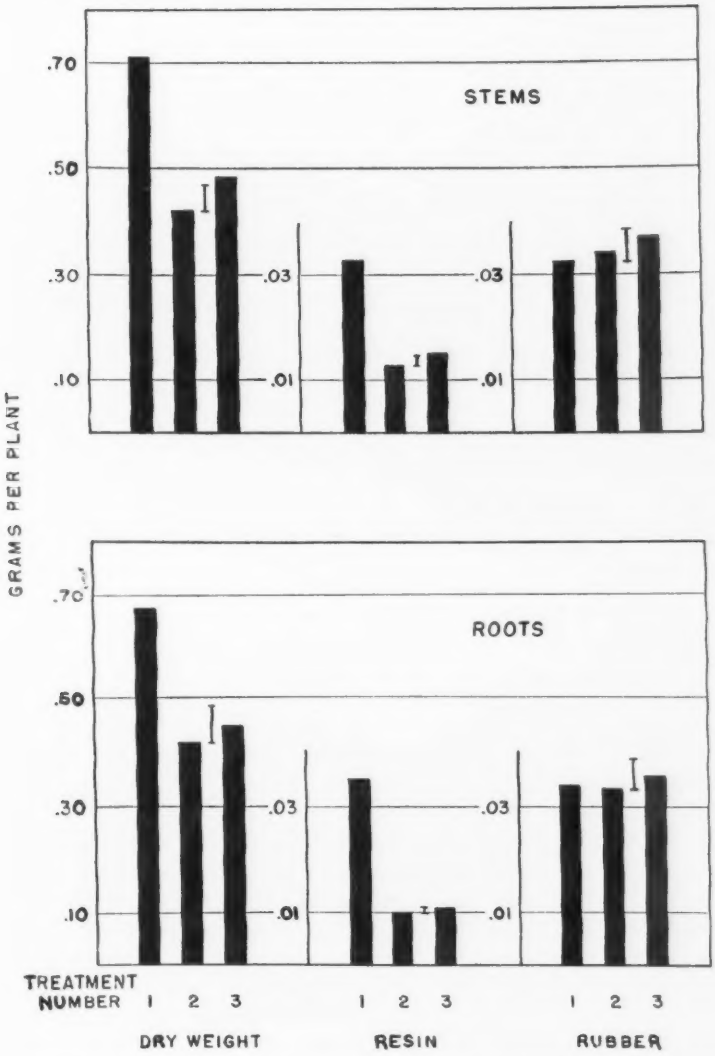


FIG. 2.—Mean dry weight, weight of resin, and weight of rubber per plant of stems and roots of guayule plants at start of experiment (treatment 1) and after being grown with repeated defoliation for some months (treatments 2 and 3). Difference required for significance between all three means is represented by length of bar between treatments 2 and 3.

ed out that the resins include all acetone-soluble compounds, and hence many types of materials, and that some of these slowly volatilize. It may be that at least some of this resin loss was due to such volatilization without any replacement rather than to utilization of the resins as a food reserve.

The fact that the resins greatly increased in the original tissues of the foliated plants further points out the degree of exhaustion of the reserves that existed in the repeatedly defoliated plants.

TRAUB (9) showed a loss of resin in the fall of the year in both foliated and repeatedly defoliated plants, plus an increase in rubber hydrocarbon that, at least in the case of the roots, exactly compensated for the resin loss. In the present experiments in the spring of the year the foliated plants showed increases in rubber and resin, while the repeatedly defoliated plants showed a decrease in resin but no such increase in rubber content as TRAUB found. This is the only way in which the results of the two experiments show marked differences; this may be related to the effects of some characteristic of the season on the plants or to the nature of the plants at the start of the experiment. Thus, the plants used in this experiment were 11 months old and had gone through one growing season in the greenhouse, while those used by TRAUB were 16 months old and had gone through two growing seasons outside.

When the new shoots started some distance below the top of the original stem, their weight was not so great as that of new shoots that arose from the very tip of the original stem; in the former plants the reduction in dry weight of the original stem on repeated defoliation was also less. This suggests that perhaps the new shoots were not able to utilize some of the reserves above as readily as they did those below their point of origin. Aside from these responses the repeatedly defoliated plants of treatments 2 and 3 did not differ from each other; and in neither treatment was there any utilization of rubber.

The effect of continued defoliation in greatly increasing the rubber percentage and in decreasing the actual amount as well as percentage of resins suggests that field defoliation prior to harvesting matured shrubs might be one method of conditioning the shrub to reduce both the milling cost and the amount of resin and acetone- and benzene-insolubles in the crude rubber obtained. This would be particularly true of the regrowth of pollarded plants in which the proportion of readily hydrolyzable compounds is much greater than in the older parts of the shrub.

Summary

1. Guayule plants were exhausted of their food reserves during the spring season of the year by repeated defoliation from January 20 to June 1. The weights per plant of free sugars, levulin, inulin, pentosans, resins, and rubber were determined in original stem and root sections at the beginning and end of the defoliating period, and the results were compared with those obtained by TRAUB (9) in a similar experiment carried out in the fall and winter months, the middle of October to the middle of January.

2. Although the food reserves (sugars, levulins, inulin, and pentosans) were very severely depleted as a result of the defoliation, from 38 to 40% of the dry weight of the original stems and roots being lost, no loss of rubber hydrocarbon occurred in these tissues.

3. It is concluded that rubber does not

serve as a food reserve and that the losses of rubber on a weight-per-plant basis reported as occurring during the spring of the year when new growth is taking place very rapidly are probably the result of sampling error or of some systematic error in harvesting that occurs at that season of the year.

POST OFFICE BOX 503
OAK RIDGE, TENNESSEE

LITERATURE CITED

1. BENEDICT, H. M.; MCRARY, W. L.; and SLATTERY, M. C. Response of guayule to alternating periods of low and high moisture stresses. BOT. GAZ. **108**:535–549. 1947.
2. ———. Unpublished data. 1948.
3. HAYES, H. K., and IMMER, F. R. Methods of Plant Breeding. McGraw-Hill Book Co., New York. 1942.
4. HOAGLAND, D. R., and ARNON, D. I. The water culture method for growing plants without soil. Calif. Agr. Exp. Sta. Circ. **347**. 1938.
5. MAY, R. H. Report on monthly increment study of 1944–45. Emergency Rubber Project, U.S. Forest Service. 1945.
6. MCRARY, W. L., and SLATTERY, M. C. The colorimetric determination of pentoses and pentosans. Arch. Biochem. **6**:151–156. 1945.
7. NICHOL, A. A., and SWENSON, M. Progress report on the third over-all indicator plot sampling. Report Emergency Rubber Project, U.S. Forest Service. 1943.
8. SPENCE, D., and MCCALLUM, W. J. The function of the rubber hydrocarbon in the living plant. Trans. Inst. Rubber Ind. **11**. 1935.
9. TRAUB, H. P. Concerning the function of rubber hydrocarbon (caoutchouc) in the guayule plant, *Parthenium argentatum* A. Gray. Plant Physiol. **21**:425–444. 1946.
10. ———. Rapid photometric methods for the determination of rubber and resins in guayule tissue and rubber in crude rubber products. U.S. Dept. Agr. Tech. Bull. 920. 1946.
11. TRAUB, H. P., and SLATTERY, M. C. Analysis of levulins, inulin, and monosaccharides in guayule. BOT. GAZ. **108**:295–299. 1946.

RELATIONSHIP OF BLUE AND ORANGE FLOWER-COLOR TYPES IN NEMESIA STRUMOSA

HERBERT PARKES RILEY

Introduction

The effect of the simultaneous presence of anthocyanins and anthoxanthins (flavones and flavonols) on flower color has been known for some time. In one of the earlier papers in his brilliant series of flower-color studies, LAWRENCE (3) showed that *Dahlia* flowers containing both deep anthocyanin and yellow flavone pigments are scarlet but that those with ivory flavone and the deep anthocyanin are magenta. Flowers with a paler anthocyanin and the yellow flavone are apricot, and those with pale anthocyanin and the ivory flavone are pale magenta. This color relationship is confirmed by mosaic flowers in which the anthocyanin is present in flecks and streaks. When the flavone ground color is ivory, the streaks and flecks are magenta, but, when the background is yellow, they are orange-scarlet. These color differences are strikingly illustrated in plate IX of a subsequent paper of LAWRENCE (4).

LAWRENCE and PRICE (6) have pointed out that, when a yellow anthoxanthin and an anthocyanin occur together, the resultant color is a blend of these two. Such a mixture would account for the apricot-scarlet tones in *Dahlia*. Ivory anthoxanthin, however, acts as a co-pigment with the anthocyanin, forming weak additive complexes that are much bluer than the anthocyanin alone. These

were described by ROBINSON and ROBINSON (13) and LAWRENCE (5). LAWRENCE and SCOTT-MONCRIEFF (7) showed that in *Dahlia* the ivory flavone acts as a strong co-pigment in flowers pigmented with cyanin, making the cyanin bluer, but that its action on pelargonin is considerably weaker. With the light anthocyanin pigmentation the ivory flavone produced a bluish-magenta color, but the flowers which lacked any flavone were rosy-magenta. With the deep anthocyanin, flowers with the ivory flavone were reddish-blue, but those with no flavone were bluish-red. The yellow flavone has no co-pigment action on either anthocyanin. A more complete account is found in BLANK's (1) review of the field of anthocyanin pigments of plants.

The writer (10) has found similar results in *Nemesia strumosa*. The flavone ground color may be orange or "white," and there may also be anthocyanin pigmentation covering the inner part of the corolla limb (11). The "white" type is not a true white in the sense of lacking flavones, for it turns yellow in ammonia. It probably corresponds, therefore, to LAWRENCE's ivory and thus acts as a co-pigment. It has been called "white," however, because it appears white phenotypically; it resembles figure 1 rather than figure 2 of LAWRENCE's plate XII (3). The anthocyanin in combination with the orange flavone results in a corolla which is Carmine, Bordeaux, Ox-blood Red (8), or some other reddish color, but in combination with the "white" background it produces a flower which is Pomegranate Purple, Amaranth Purple, Tyrian Rose, or some other color on the bluish side.

Another interesting type that was found in the writer's early material was the blue-margin flower (9). More recently, a self-blue has also been obtained. These blue types are entirely different from the "colored" type, and, because of the interaction of the anthocyanin pigmentation with the orange and white types, it seemed of interest to study this blue type in combination with both orange and white.

The source of these plants has been described (10). It is questionable whether the self-blue form sold as *N. strumosa suttoni* really belongs to that species, because the flowers were rather small and were spurred. *N. strumosa* has a short, broad, slightly bifoveolate pouch rather than a spur, although most of the other species bear a spur (2). Because the blue, spurred type crossed readily with typical *N. strumosa*, the species question was ignored.

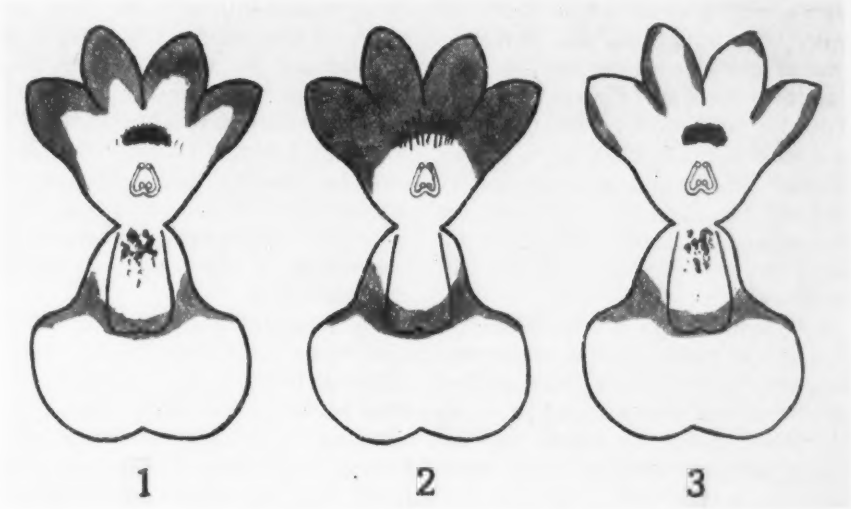
Observations

BLUE-MARGIN.—The blue-margin type resembles the white-flowered type which the writer (10) has designated as the standard, except for some blue coloration on the upper lip of the corolla and on the lower lip at the sides of the throat and for the presence of less orange pigmentation in the lower part of the tube. The extent of the pigmentation on the upper lip varies according to other genes which are present. In *cc oo* plants which have the eyebrow gene (12) the blue color appears to be restricted to a margin 1 or 2 mm. wide which extends around the edges of the upper lips (fig. 1). In *cc oo* plants which are homozygous for the recessive non-eyebrow gene the blue appears to cover all the upper lips and to extend down over the few thin lines which replace the heavy eyebrow in *ee* plants (fig. 2). The blue color on both lips may vary somewhat but generally is close to Chicory Blue, Lavender-Violet, Mauve, or Manganese Violet of RIDGWAY (8).

When a blue-margin flower is exposed

to fumes of ammonia, the blue color turns somewhat more blue and sometimes greenish. In nitric acid the blue turns reddish, and in sulfur dioxide it is almost all bleached out.

In the pedigrees derived from the original lot of seeds the lower part of the tube of all blue-margin plants was straw color to white instead of orange as in all other types. If the eyebrow gene was present, cally the bicolor type described previously by the writer (11). In both the upper lip is deep purple, and the lower lip is paler and nearer a rose type. The upper lip of the purple-blue-margin type is more unevenly colored, appearing usually as small irregular patches of dark purplish-blue interspersed in the usual purple pigmentation. The tube of the purple-blue-margin type is straw or at



FIGS. 1–3.—Diagrams of flowers of *Nemesia strumosa*. Each flower has been cut along both sides of tube and then flattened out. Fig. 1, blue-margin with the dominant eyebrow. Fig. 2, blue-margin with the recessive non-eyebrow. Fig. 3, blue-margin reduced. In all figures the shaded area is blue.

the black or very dark purple dots on the inner part of the tube were nearer a reddish-purple. In the pedigrees from the more recent lots of seeds, however, some of the blue-margin plants had two very small patches of yellow or orange on the lower part of the tube near the pouch, and in some plants there was a single and somewhat larger patch. In no blue-margin plant was the tube so extensively orange as in the non-blue-margin types.

Plants with the color gene *C* in addition to blue-margin resemble phenotypi- most has only small orange areas, whereas that of the bicolor type is orange. The two types, however, are readily confused.

Blue-margin appears to be recessive to white. Crosses between two blue-margin plants in four families gave 113 blue-margin and 0 non-blue-margin. One family from a cross between a blue-margin and a white contained 67 white and 0 blue-margin. Nine F_2 families segregated into 220 non-blue-margin and 74 blue-margin. The recessive nature of blue-margin is confirmed by a few plants in which a

blue sectorial chimaera appeared on the upper lip of white-flowered plants in families that were known to contain plants heterozygous for blue-margin.

Each F_2 family listed above had one self-sterility allele common to the two parents. The close approximation to 3:1 ratios indicates an absence of linkage between the blue-margin and *s* genes. One family in which there was a self-sterility allele common to the two parents arose from a cross between a blue-margin female and a white male and segregated into 28 non-blue-margin and 29 blue-margin. If there were linkage with the *s* genes, the ratio would not be expected to be so close to 1:1. The absence of linkage between blue-margin and self-sterility confirms the distinction between the blue-margin and bicolor phenotypes, for the bicolor gene is linked with the self-sterility alleles.

Four families from a cross between a *Cc* and a *cc* plant, both of which were heterozygous for blue-margin, segregated into 82 colored non-blue-margin : 19 colored blue-margin : 77 white : 23 blue-margin, and one backcross family segregated into a ratio of 15 : 14 : 13 : 15. These crosses indicate that there is no linkage between *C* and the blue-margin gene.

Two families from the original material indicate that there is no linkage between eyebrow and blue-margin, but the newer material was not tested because the distinction between eyebrow and non-eyebrow was not so clear cut in some of these plants. One family from a cross between a colored and a white each heterozygous for blue-margin and eyebrow segregated into 13 colored : 3 colored blue-margin : 7 white eyebrow : 3 white non-eyebrow : 4 blue-margin eyebrow : 0 blue-margin non-eyebrow. The numbers are small but suggest no linkage. A cross between two whites heterozygous for both genes segregated into 51 white eyebrow : 10 white non-eyebrow : 27 blue-margin eyebrow : 5 blue-margin non-eyebrow. Although this ratio is not good, it does not indicate any linkage.

One family indicates that there might be linkage between blue-margin and the glass-green gene. The parents were a white and a glass green, and both were heterozygous for blue-margin. The family segregated into 39 white : 24 glass green : 1 blue-margin : 31 glass green, blue-margin. In the glass-green–blue-margin type the upper lip was like the usual blue-margin type, but the lower lip was glass green, not white. This ratio could have resulted from a cross between a heterozygote in the coupling phase and a recessive glass green heterozygous for blue-margin if there were about 4% crossing over.

The relationship between blue-margin and orange is not clear. Of the original material, two families resulted from a cross between a blue-margin female and an orange male. These two families were related, for the orange male parent of one family was the paternal grandmother of the second. One of the families segregated into 26 orange and 17 blue-margin and the second contained 9 orange and 12 blue-margin plants. The relationship is summarized in table 1. Several explanations for this behavior are possible and will be discussed later in this paper.

BLUE-MARGIN REDUCER.—Although the margin of blue color on most phenotypically blue-margin plants is fairly uniform, some plants have a margin that is broken or much reduced and very irregular. The appearance of such plants in only a few families and only in ones that are genetically related suggests that the reduced condition of the margin is inherited and as a recessive. This character

(fig. 3) is difficult to score. In three families from apparently heterozygous parents the blue-margin–eyebrow plants segregated into 39 with normal margins and 4 with reduced margins. Two backcrosses gave 17 normal and 12 reduced. The reducer gene has no effect on non-eyebrow–blue-margin plants. The symbol of this gene shall be *br*.

BLUE.—The blue type differs from blue-margin in that the blue color extends over both lips of the corolla. The blue is RIDGWAY's Pale Blue-Violet, Pale Violet Blue, or sometimes Pallid Violet Blue. The lower part of the tube is white with a small spot of orange near the rather long spur.

Blue behaves as a simple recessive to white. A cross between a blue and a white resulted in the production of 11 whites. Six families from crosses between various F_1 plants consisted of 80 whites and 26 blues, but the segregation was good in only four of the families. A backcross between a blue and a white heterozygous for blue gave 11 white and 9 blue. When a blue was crossed with a yellow (*yy*), the F_1 was white, showing that the two genes were not alleles. Segregation in later generations was not studied, because the yellows were too difficult to identify.

ALLELIC RELATIONSHIP.—The similarity in color between blue-margin and blue made it very desirable to test them together. Both are recessive to white and should produce white when crossed, unless they are alleles. Unfortunately, no known homozygous blue-margin plant was available at the same time as blues. The earlier material contained no blues, and the later material contained only one blue-margin plant, and that was obtained along with many plants with orange flowers from a seed packet marked "Orange." When this blue-margin plant was crossed with a blue, three blue-margin plants were obtained. Three later families also arose from a cross between a blue and a blue-margin and contained 9 blue-margins and 8 blues. The fact that blue-margin and blue when crossed do not produce white indicates that they are alleles. Which, then, is dominant over the other? One family arose from a cross between two blue-margins heterozygous for blue and segregated into 15 blue-margin and 8 blue. When a white-flowered plant heterozygous for blue-margin was crossed with

TABLE 1

SEGREGATION IN THE TWO FAMILIES (3322 AND 3377) OF THE ORIGINAL MATERIAL WHICH RESULTED FROM CROSSES BETWEEN A BLUE AND AN ORANGE AND THE FAMILIES THAT GAVE RISE TO THEIR PARENTS. FOR GENE SYMBOLS SEE TEXT

FAMILY	PARENTS	GENOTYPES OF PARENTS	PHENOTYPES OF OFFSPRING			
			Colored	Orange	White	Blue-margin
3112	3012(11)×3012(8)	$cco^{bm}\times cco^{bm}$	0	0	61	32
3103	3001(6)×3001(22)	$ccOO\times CcO^{bm}$	39	44	0	0
3322	3112(9)×3103(52)	$cco^{bm}{}^{bm}\times ccO^{bm}$	0	26	0	17
3222	3112(6)×3112(3)	$cco^{bm}{}^{bm}\times cco^{bm}{}^{bm}$	0	0	0	41
3224	3103(52)×3103(1)	$ccO^{bm}\times ccOO$	0	27	0	0
3377	3222(7)×3224(2)	$cco^{bm}{}^{bm}\times ccO^{bm}$	0	9	0	12

a blue, the offspring segregated into 39 white and 27 blue-margin, and there were no blue-flowered plants. When two whites were crossed, one of which was heterozygous for blue-margin and the other for blue, there were 51 whites and 24 blue-margins and again no blues. These crosses indicate that there are at least three alleles. White is the top dominant, and blue-margin is dominant over blue but recessive to white. Further evidence that blue is recessive to blue-margin is afforded by a heterozygous blue-margin plant that had a blue chimaera on the lower lip.

As table 1 shows, two families from crosses between an orange and a blue-margin segregated into approximately equal numbers of orange and blue-margin types and nothing else. There are several possible explanations for these results. Blue-margin and orange might be alleles, with the orange parents heterozygous. Blue-margin and orange might be on separate chromosomes, with orange epistatic and the orange parents homozygous for the blue-margin gene. The blue-margin and orange genes might be very closely linked and in the coupling phase. With the addition of the blue gene, it was hoped that some further evidence for the most probable explanation would be obtained.

In two families an orange crossed with a blue-margin resulted in 10 orange plants, and in two other families a red (anthocyanin plus orange) and a blue-margin resulted in 33 red-flowered plants. Two families from a blue and an orange parent contained 22 orange plants. One family came from two red-flowered parents and segregated into 20 red : 9 purple–blue-margin : 9 orange : 2 blue-margin, which suggests that orange and blue-margin might be allelic. The parents of both the red-flowered parents were a red and a blue-margin. Five F_2 families from a cross between an orange and a blue consisted of 126 orange and 36 blue, indicating an allelic relationship between these two types.

Figures 4 and 5 illustrate the principal families that show a relationship among these genes. Assume that the genes are on separate chromosomes with orange epistatic. For families 4713, 4716, 4717, 4718, and 4719 to contain only orange and blue types, plants 4433 (1) and (7) and 4434 (1), (2), (3), and (4) would all have to be homozygous for the recessive blue gene. If 4394 (2) had a blue-margin gene, half the offspring would be expected to have a blue-margin gene, but none of these six plants did. On the other hand, if 4394 (2) is homozygous for blue, plant 4431 (1) would be heterozygous for blue, and in families 4712 and 4715 one-half of the non-orange offspring should be blue. This relationship is not conclusive, but the probabilities are against a two-factor scheme.

Two other explanations are (*a*) that orange, white, blue-margin, and blue form a series of multiple alleles with dominance in that order and (*b*) that orange is at a separate locus from the blue–blue-margin locus, that all orange plants so far tested have the dominant non-blue, non-blue-margin gene coupled with the dominant orange, and that there is complete linkage between the orange and blue loci. The problem of deciding between these two hypotheses is the same as that faced in similar situations in other organisms. About the only way to decide is to look for cross-over types that would emerge from a cross between an orange plant heterozygous and in the coupling phase and a blue or blue-margin. A non-orange, non-blue, non-blue-margin would be white and readily identifiable. An orange-blue or orange–blue-

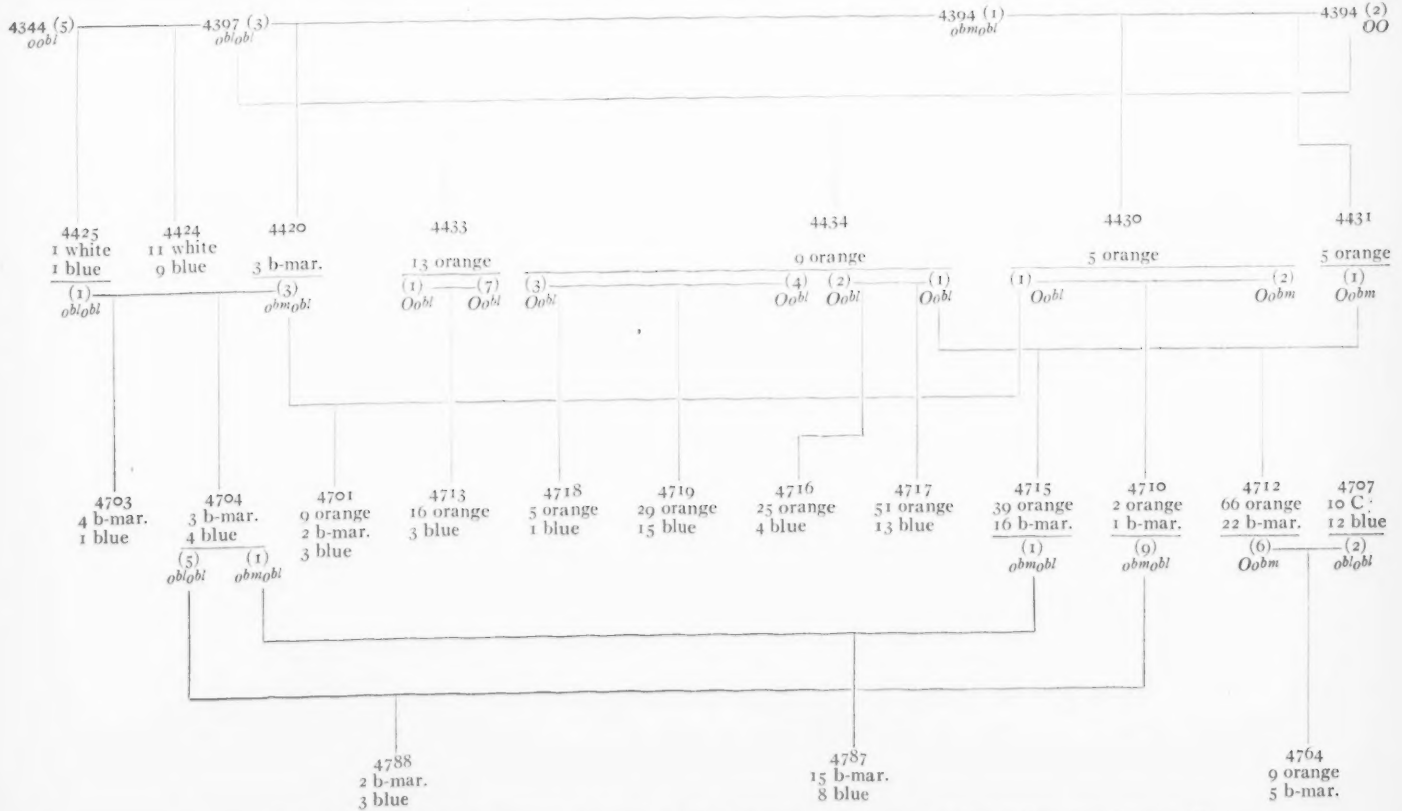

4344 (5) oobl
4397 (3) obl obl
4394 (1) obm obl
4394 (2) OO
4425 1 white 1 blue (1) obl obl
4424 11 white 9 blue
4420 3 b-mar. (3) obm obl
4433 13 orange (1) Oobl (7) Oobl
4434 9 orange (3) Oobl (4) Oobl (2) Oobl (1) Oobl
4430 5 orange (1) Oobl (2) Oobm
4431 5 orange (1) Oobm
4703 4 b-mar. 1 blue
4704 3 b-mar. 4 blue (5) obl obl (1) obm obl
4701 9 orange 2 b-mar. 3 blue
4713 16 orange 3 blue
4718 5 orange 1 blue
4719 29 orange 15 blue
4716 25 orange 4 blue
4717 51 orange 13 blue
4715 39 orange 16 b-mar. (1) obm obl
4710 2 orange 1 b-mar. (9) obm obl
4712 66 orange 22 b-mar. (6) Oobm
4707 10 C 12 blue (2) obl obl
4788 2 b-mar. 3 blue
4787 15 b-mar. 8 blue
4764 9 orange 5 b-mar.

FIG. 4.—Pedigree chart showing inheritance of orange, white, blue-margin, and blue in families lacking the color gene, *C*.

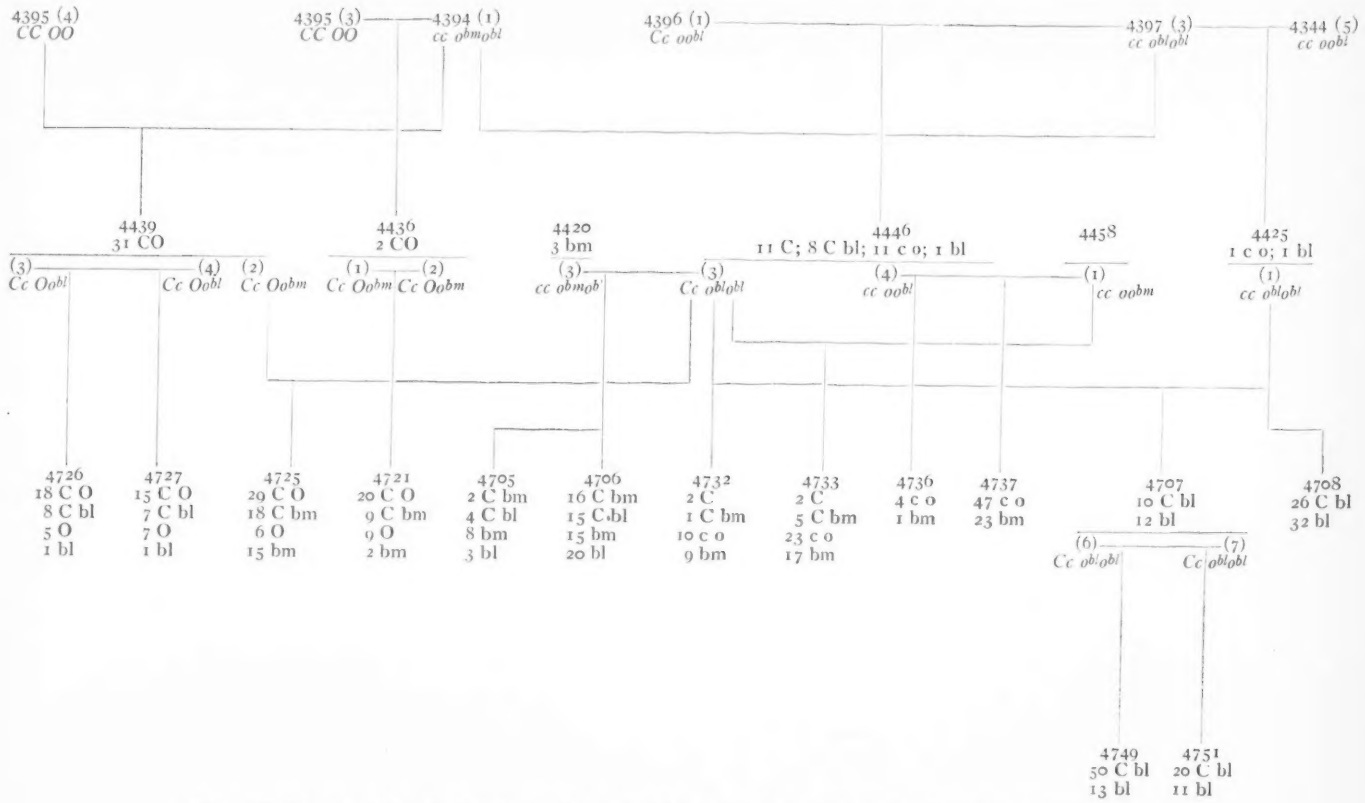


FIG. 5.—Pedigree chart showing inheritance of orange, white, blue-margin, and blue in families with the color gene, *C*.

margin would probably represent a new phenotype not previously found but conceivably would be a blend of the orange flavone and the blue anthocyanin. In all the families studied no white-flowered plants and no new phenotypes that might be identified as the other cross-over type were found. In the absence of any conclusive evidence, the hypothesis of multiple alleles is tentatively adopted.

The hypothesis of a series of four alleles with orange dominant over the other three, white dominant over the blue types, blue-margin dominant over blue, and blue recessive to the other three will explain satisfactorily all the families listed in figures 4 and 5. Various crosses involving orange are summarized in table 2, and these also are in accord with the hypothesis. Until there is evidence that the hypothesis of multiple alleles is not the correct explanation, the following symbols will be used: *O* for orange, *o* for white, o^{bm} for blue-margin, and o^{bl} for blue. These supersede the symbols of an earlier paper (9). Probably, according to the rules, the symbol for blue should form the basis of the system, and the other symbols should be built up from that one by superscripts. Since the symbols *O* and *o* have already been published for orange and white, respectively (10), they might just as well be retained and the others be based on them.

That the blue and orange genes do not combine to form a third phenotype is suggested by one plant of family 4764. As figure 4 indicates, the orange-flowered plants of this family were heterozygous for blue. On the lower lip of one flower of an orange-colored plant there was a wide blue sector. This striking type was perhaps produced by the somatic nondisjunction of the chromosome bearing the orange gene.

Two families gave a peculiar ratio of colored to noncolored. They are 4732 and 4733 (fig. 5). One parent was a colored-blue type and the other was a white of unknown ancestry, designated 4458 (1) in the pedigree chart. The writer desired to cross some of the known types with a white, but apart from 4446 (4) no white-flowered plants of known ancestry were in good condition at the time. The white plant which was used was from one of the commercial seed packets and proved to be heterozygous for blue-margin. The

TABLE 2

CROSSES AMONG VARIOUS ORANGE, BLUE-MARGIN, AND BLUE PLANTS; GENOTYPES OF THE PARENTS ARE EXPRESSED IN SYMBOLS BASED ON THEORY OF MULTIPLE ALLELES. ONLY CROSSES IN WHICH ONE OR BOTH PARENTS ARE ORANGE ARE INCLUDED

Genotypes of parents	Number of families	Offspring					
		C O	*C bm*	*C bl*	*O*	*bm*	*bl*
$cc\ OO \times cc\ o^{bm}o^{bl}$	2	.....	.....	.....	10	0	0
$CC\ OO \times cc\ o^{bm}o^{bl}$	2	33	0	0	.....	.....	.....
$Cc\ Oo^{bm} \times Cc\ Oo^{bm}$	1	20	9	.....	9	2	.....
$cc\ Oo^{bm} \times cc\ Oo^{bl}$	3	.....	.....	.....	107	39	0
$cc\ Oo^{bm} \times cc\ o^{bl}o^{bl}$	1	.....	.....	.....	9	5	0
$Cc\ Oo^{bm} \times Cc\ o^{bl}o^{bl}$	1	29	18	0	0	15	0
$cc\ Oo^{bl} \times cc\ o^{bm}o^{bl}$	1	.....	.....	.....	9	2	3
$cc\ OO \times cc\ o^{bl}o^{bl}$	2	.....	.....	.....	22	.....	0
$cc\ Oo^{bl} \times cc\ Oo^{bl}$	5	.....	.....	.....	126	.....	36
$Cc\ Oo^{bl} \times Cc\ Oo^{bl}$	2	33	.....	15	12	.....	2

ratio of colored to white (10:59) in families 4732 and 4733 indicates that the white-flowered plant was heterozygous for an anthocyanin inhibitor.

Summary

1. Two flower-color types in *Nemesia strumosa* are blue-margin and blue. In blue-margin plants possessing the eyebrow gene, there is a band of pale blue color on the inside of the corolla limb around the margins of the lobes of the upper lips, and some blue color is present on the inside of the lower lip at the sides of the tube, frequently extending over the throat. In blue-margin plants homozygous for the allele of eyebrow, the blue color covers practically the entire surface of the inner part of the upper lip. In the blue type both lips are blue on the inner surface. In both types there is little or no orange pigmentation in the lower part of the tube.

2. When combined with the color gene *C*, blue forms a more bluish-purple color that is often hard to identify, but blue-margin forms a type which is purple on the upper lip and a shade of rose on the lower. This type resembles the bicolor type but can usually be differentiated by the lesser amount of orange in the tube.

3. Blue and blue-margin seem to form a series of multiple alleles with orange and white with orange the top dominant, white dominant to the blue types, blue-margin dominant to blue, and blue recessive to the other three types. The genes are designated, respectively, *O*, *o*, o^{bm}, and o^{bl}.

4. A recessive gene for blue-margin reducer, *br*, reduces the width of the margin in blue-margin, eyebrow plants. In some places the margin is broken.

5. There is indication of the presence of a dominant gene that acts as an anthocyanin inhibitor.

University of Kentucky

LITERATURE CITED

1. Blank, F. The anthocyanin pigments of plants. Bot. Rev. **13**:244–317. 1947.
2. Hiern, W. P. Flora Capensis, ed. W. T. Thiselton-Dyer. Vol. **4**. 1904.
3. Lawrence, W. J. C. The genetics and cytology of *Dahlia* species. Jour. Genet. **21**:125–159. 1929.
4. ———. The genetics and cytology of *Dahlia variabilis*. Jour. Genet. **24**:257–306. 1931.
5. ———. Interaction of flavones and anthocyanins. Nature **129**:834. 1932.
6. Lawrence, W. J. C., and Price, J. R. The genetics and chemistry of flower colour variation. Biol. Rev. **15**:35–58. 1940.
7. Lawrence, W. J. C., and Scott-Moncrieff, R. The genetics and chemistry of flower colour in *Dahlia:* a new theory of specific pigmentation. Jour. Genet. **30**:155–226. 1935.
8. Ridgway, R. Color Standards and Color Nomenclature. Washington, D.C. 1912.
9. Riley, H. P. Interaction of genes for flower color in *Nemesia strumosa*. Genetics **25**:133 (abstr.). 1940.
10. ———. Inheritance of orange and yellow flower color in *Nemesia strumosa*. Bull. Torr. Bot. Club **72**:435–453. 1945.
11. ———. Inheritance of the main anthocyanin pigmentation and of some of its patterns in flowers of *Nemesia strumosa*. Bot. Gaz. **107**:32–52. 1945.
12. ———. Inheritance of the "Eyebrow" gene in *Nemesia strumosa*. Jour. Hered. **39**:170–172. 1948.
13. Robinson, G. M., and Robinson, R. A survey of anthocyanins. I. Biochem. Jour. **25**:1687–1705. 1931.

EFFECT OF SULFANILAMIDE ON MITOTIC DIVISION IN POLLEN GRAINS OF EPHEDRA[1]

P. N. MEHRA

Introduction

EIGSTI (3) reported that "mitotic irregularities" were induced in the pollen tube of *Tradescantia occidentalis* in a very dilute concentration of sulfanilamide (1:10,000) and that higher concentrations inhibited pollen-tube formation. TRAUB (9) found that, when seeds and cuttings of bean (*Phaseolus vulgaris*) were treated with a 0.5% solution of sulfanilamide, plants were produced which showed many variations indicative of polyploidy, though he made no cytological confirmations. In the same paper he reported that exposure of the root tips of *Allium* to a 0.5% solution of the chemical led to the formation of cells with tetraploid constitution and some with two free nuclei, but he did not study the mechanism involved in the process.

PETERS (8) tested the effect of a 0.5% aqueous solution of sulfanilamide on root tips of *Allium cepa*. He found that the cells already in metaphase or anaphase at the onset of the treatment with sulfanilamide completed nuclear division but that in those at prophase the spindle was inhibited and tetraploid nuclei were organized. Often micronuclei were formed by reversion of different groups of chromosomes in some cells. He concluded that "sulfanilamide (0.5%), unlike colchicine (0.1%), inhibits the entrance of cells into mitosis."

FULLER (4) tried the effect of various concentrations of sulfanilamide in aqueous solution and of a 0.1% strength of five other sulfa- compounds (sulfadiazine, sulfaguanidine, sulfamerazine, sulfapyridine, and sulfathiazole) on mitosis in root tips of *A. cepa*. In all cases there was reduced mitotic activity and suppression in visible root elongation. In the case of sulfa- compounds other than sulfanilamide normal mitosis was observed, and the spindle was never disrupted. This was ascribed to the low solubilities of the chemicals. In 0.5% sulfanilamide, even after 96 hours of treatment, a few cells exhibited a continuation of normal mitosis, although in most cases the inhibition of the spindle with resultant tetraploidy was seen. This concentration of the chemical brought about a marked shortening of the late prophase chromatids. The lower concentrations did not result in collapse of the spindle.

In the present investigation the effect of sulfanilamide has been tried on the mitotic division of the body nucleus in the germinating pollen grains of four species of the genus *Ephedra*, viz., *E. foliata* Boiss., *E. sinica* Stapf, *E. saxatilis* Royle, and *E. intermedia* Schrenk and Meyer. The haploid chromosome number for *E. foliata* is seven; for the other three species, fourteen (7). The mature pollen grain in *Ephedra* contains a large tube

[1] [This paper was apparently received in the editorial offices of the BOTANICAL GAZETTE in March or April, 1946, but, unfortunately, was incorrectly filed and did not come to the attention of the present editor until late in 1948. In the meantime papers by PETERS (8) and by FULLER (4) had appeared on the effect of sulfanilamide on mitosis in root tips of *Allium cepa*. Dr. MEHRA has therefore revised his paper in order to make comparisons with their observations.—EDITOR.]

nucleus at one end, a body nucleus in the center of a body cell located in the middle of the grain, a much condensed stalk nucleus at the periphery of the body cell—usually on the end opposite to the tube nucleus—and remnants of two vegetative cells.

Under natural conditions the pollen grains fall on the mucilaginous secretion that oozes from the end of the micropylar tube in a ripe ovule. They are later carried into the micropylar canal and then into the pollen chamber, where they germinate. This secretion has been utilized artificially in bringing about the germination of the pollen grains on glass slides kept in a moist chamber. The exine is ruptured after a short time, and the body nucleus undergoes mitotic division, ultimately organizing two male nuclei. The total period of division varies from 3 to 8 hours, depending on the temperature. At ordinary room temperature in April the total period is about 3 hours, while earlier in the season it is relatively longer.

The problem has been investigated from two aspects: (*a*) the direct immediate effect of the chemical when applied at different phases of mitosis and (*b*) the alteration in the mitotic cycle brought about when pollen is germinated in its presence.

Observations

EFFECT OF SULFANILAMIDE APPLIED AT DIFFERENT PHASES OF MITOSIS

In these experiments the effect of only one concentration, 0.5%, was tried on the four species. The pollen grains were germinated on the normal natural secretion until the desired stages were realized, as verified from parallel control cultures started simultaneously and stained with acetic-iodine-green or acetocarmine for observation of the progress of mitosis. At the desired stage 1% sulfanilamide in distilled water was thoroughly mixed in equal amount with the secretion containing the germinating grains, thereby bringing the sulfanilamide concentration of the mixture to 0.5%. Fixations were made after 5 and 15 minutes, this brief period being allowed for the chemical to effect its entry into the grains. Smears were prepared after fixing in Allen's modification P.F.B.15 of Bouin's fixative, by the method already described (**7**). These were stained with Haidenhain's iron-alum haematoxylin.

The effect of the 0.5% concentration of sulfanilamide was exactly the same as that of colchicine already reported (**6**). The spindle immediately disappeared at all stages in the mitotic cycle, i.e., metaphase, anaphase, or telophase. In the metaphase preparations of many grains fixed 5 minutes after application of the chemical, the original position and orientation of the metaphase chromosomes was discerned, with their kinetochores in the same level, though the chromosomes were more or less loosened after the loss of the spindle mechanism. Soon, however, this regular arrangement was lost, for in grains fixed 15 minutes after application of the chemical the diplochromosomes were irregularly scattered in numerous ways within the boundary of the body cell. Similarly, in grains in anaphase fixed 5 minutes after application of sulfanilamide, it was seen that, although the spindle had disappeared, in many grains the original anaphasic disposition—the daughter chromosomes in two groups with the orientation of their kinetochores toward the respective poles—was retained for a short while. In the absence of the spindle the members in each of the two groups were in a more or less loosened state. Soon this regular arrangement was lost, and the daughter chromosomes became irregularly scattered, as

was evidenced in anaphase preparations fixed 15 minutes after application of the chemical.

Evidently the primary effect of sulfanilamide is to bring about an immediate collapse of the spindle mechanism. Upon effecting entry, it seems to cause excitations in the cytoplasm, thereby disturbing the equilibrium of the latter and resulting in the collapse of the spindle. The irregular distribution of the metaphase and anaphase chromosomes is ascribed to continued excitations in the cytoplasm in which the chromosomes are dispersed after the collapse of the spindle.

These observations are in contrast to those of PETERS, who found in the root tips of *A. cepa* treated for 3 hours in sulfanilamide that the cells that were at metaphase or anaphase at the beginning of the treatment completed nuclear division but did not form a cell plate.

When the chemical acts on cells in which nuclear division is at telophase, the spindle simply collapses, but two nuclei are organized in the usual manner from the two telophasic groups of chromosomes. If the two nuclei are already organized and the spindle is still present, the latter simply disappears, leaving the two free nuclei in the cytoplasm of the body cell.

The presence of two free and equal nuclei in some cells of root tips exposed to sulfanilamide, as observed by TRAUB (9), may be due to the effect of the chemical in the cells at telophase.

EFFECT ON GRAINS GERMINATED IN SECRETION CONTAINING SULFANILAMIDE

In these experiments the grains were germinated in the mucilaginous secretion to which sulfanilamide had been added. Fixations were made at definite intervals, followed by smearing and staining.

Three concentrations of sulfanilamide were tested, 0.2%, 0.4%, and 0.5%. In making a particular test concentration, a certain quantity of natural secretion was thoroughly mixed with an equal quantity of sulfanilamide solution of appropriate strength in distilled water.

A 0.2% concentration had absolutely no effect on the division mechanism of the body nucleus. All the successive stages from the prophase to the formation of two male nuclei occurred in the normal manner.

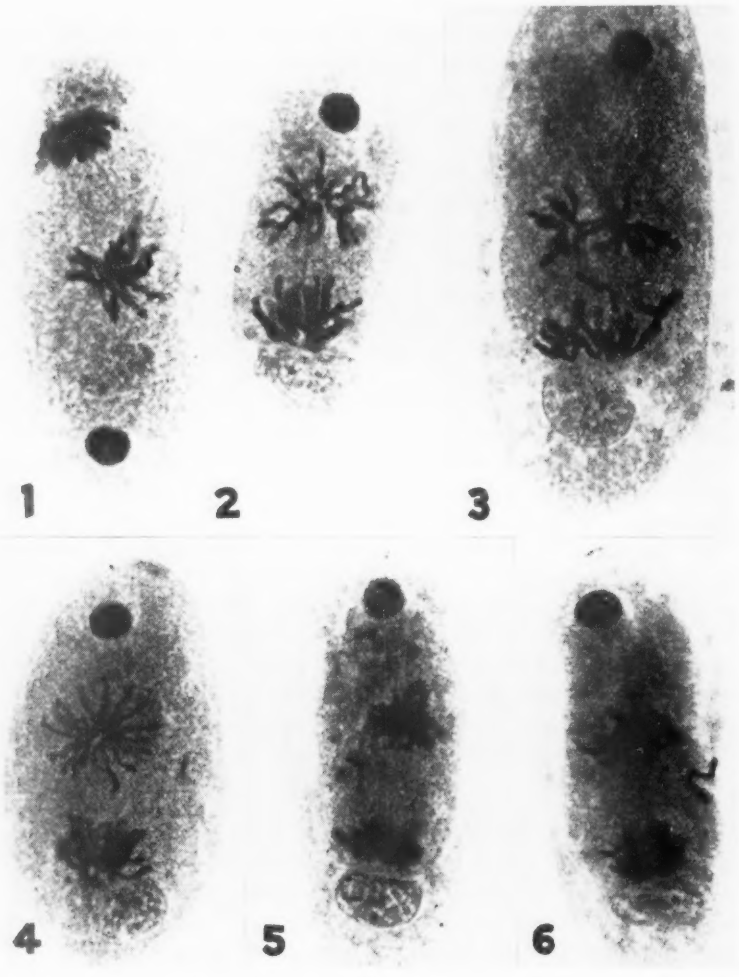
A 0.4% concentration was just at the critical level for obtaining a paralyzing effect on the spindle mechanism. Although in most of the grains mitosis proceeded normally (figs. 1–4), in some cases a chromosome from one of the daughter groups lost its attachment to the spindle and was extruded (figs. 5, 6).

A 0.5% strength of sulfanilamide was fully effective in bringing about the disturbance in the division mechanism of the body nucleus.

During the course of normal mitosis, after the disappearance of the nuclear membrane at the end of the prophase, the as-yet-elongated diplochromosomes appear in a polarized state; their free arms point toward the stalk-nucleus end of the grain and their kinetochores are directed toward the opposite end, evidently retaining the position they occupied in the previous mitosis. They then become arranged on the equator of the spindle and in the meantime undergo contraction, decreasing in length until the final metaphase stage, just before the separation of the daughter chromosomes.

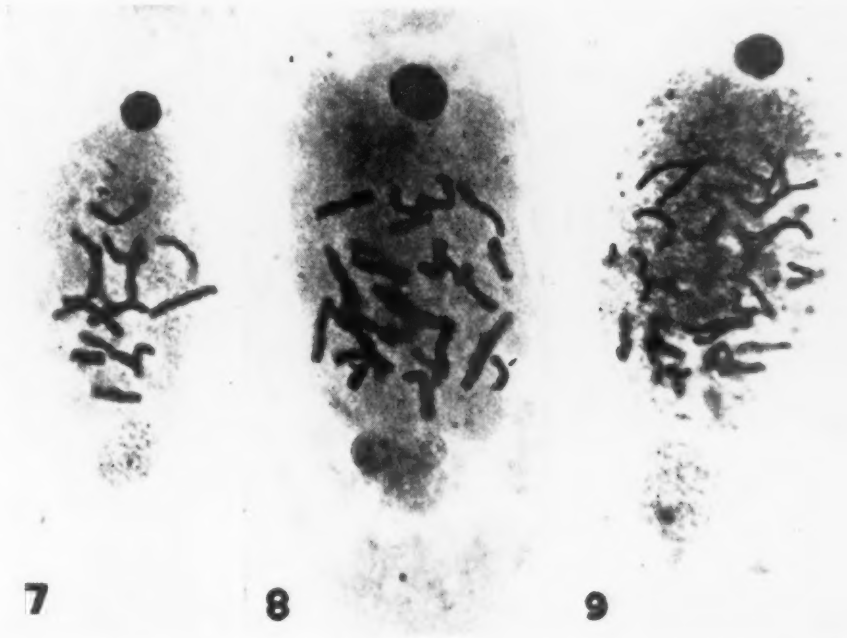
In grains germinated in 0.5% sulfanilamide, the earlier stages, until the disappearance of the nuclear membrane at the close of the prophase, were the same as in normal mitosis. Since the spindle never developed, however, the elongated

diplochromosomes became dispersed in the cytoplasm of the body cell (figs. 7, 8, 10, 17, 20). The chromosomes then became more or less straightened. Satellites and secondary constrictions were observed in the same fashion as in normal untreated preparations. Two sat-chromosomes may be seen in the complement of *E. saxatilis* in the grain shown in figure 17. One of these is clearly visible at *S*, while the other is out of focus. Both are separately illustrated in figure 23. The primary constrictions stand out prominently as in material treated with colchicine (**6**). Chromosomes underwent contraction simultaneously by spiralization of the chromonemata of their constituent chromatids. This process was



FIGS. 1–6.—Pollen grains of *E. intermedia* germinated in secretion containing 0.4% sulfanilamide. Figs. 1–4, showing normal mitosis. Figs. 5, 6, showing one daughter chromosome extruded from spindle zone.

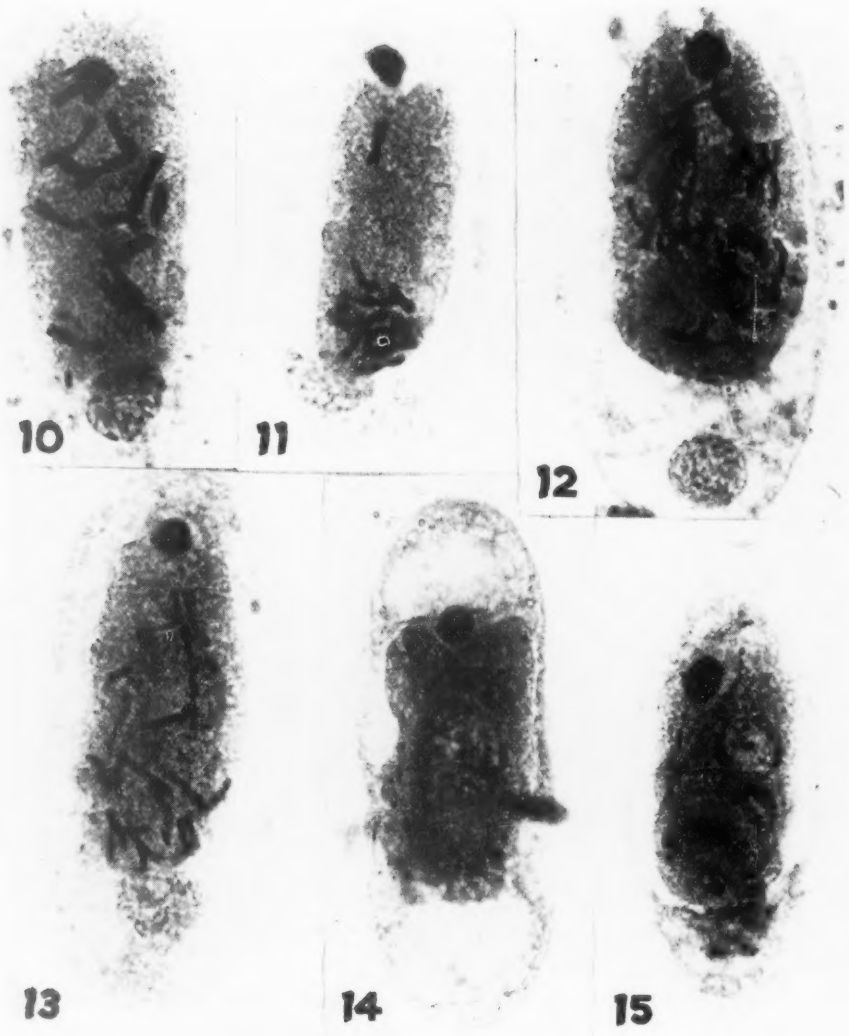
comparable to that which occurs in normal mitosis in the course of the development of the full metaphase. In the meantime the chromosomes were scattered in numerous ways. In some cases they became bunched in one or more groups or were far apart. In other cases only one metaphase chromosome was widely and later extended to the region of the centromere. Each pair of daughter chromosomes for sometime laid parallel (fig. 18), but later this disposition was lost owing to excitation movements in the cytoplasm (figs. 9, 12, 13). In well-fixed preparations the spiralization of the chromatic elements of the daughter



FIGS. 7–9.—Pollen grains of *E. intermedia* germinated in secretion containing 0.5% sulfanilamide. Fig. 7, diplochromosomes widely scattered after spindle inhibition. Fig. 8, same as fig. 7 but in diploid grain with twenty-eight diplochromosomes. Fig. 9, daughter chromosomes separated in diploid grain; in a few cases they are still close together.

drifted, while all the rest formed a separate group (fig. 11).

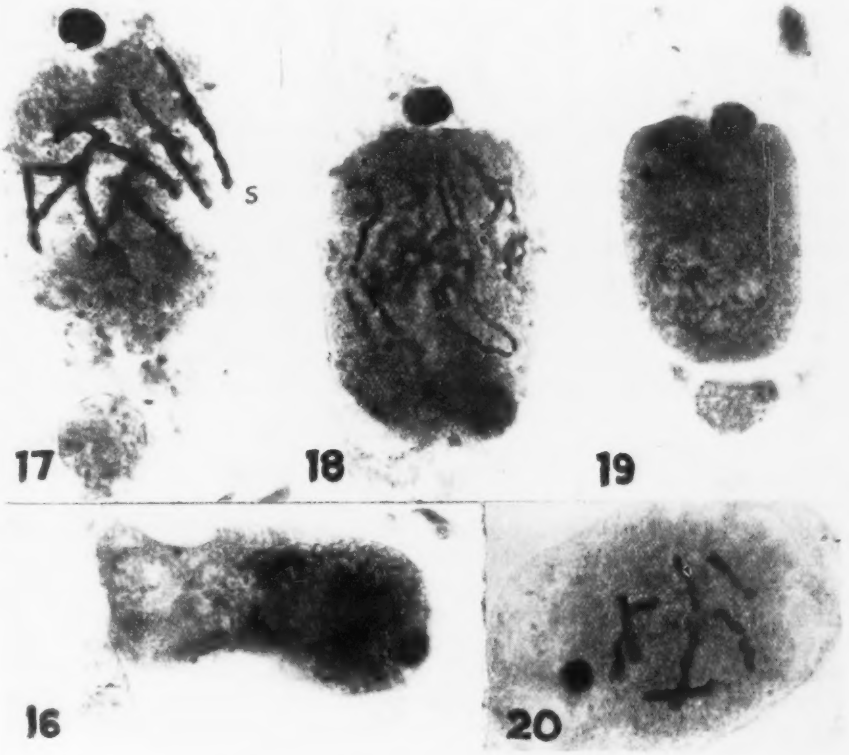
During the different stages of the contraction process the daughter chromosomes separated from each other. This separation occurred in many cases even when the chromosomes had not yet attained the size which they possess at full metaphase during the course of normal mitosis. It occurred first along the arms chromosomes may be clearly seen. As a rule the splitting of the daughter chromosomes necessarily occurred before their entrance into the resting stage, but there were a few exceptions. All the chromosomes of the complement were observed to undergo splitting simultaneously. Since the contraction of the metaphase chromosomes and their splitting occurred in the absence of the spindle



FIGS. 10–15.—Pollen grains of *E. sinica* germinated in secretion containing 0.5% sulfanilamide. Fig. 10, spindle inhibited, diplochromosomes widely scattered. Fig. 11, chromosomes grouped together after spindle inhibition except for one which has wandered away. Figs. 12, 13, daughter chromosomes separated. Fig. 14, single diploid restitution nucleus. Fig. 15, three nuclei organized from scattered chromosome groups. Cf. fig. 21.

mechanism, it may be concluded that both of these processes are an inherent property of the chromosomes themselves and have nothing to do with the spindle mechanism.

These events were followed by steps in the formation of nuclei. The number of nuclei organized was invariably dependent on the distribution of the daughter chromosomes within the space of the body cell. When the chromosomes were close together, as happened quite frequently, a single restitution nucleus was organized with a circular outline. Such nuclei were quite dense and possessed a definite membrane (figs. 14, 19). They were sharply marked off from the cytoplasm. Since all chromosomes entered into their constitution, such nuclei were diploid. If the daughter chromosomes were somewhat scattered, a diploid restitution nucleus resulted which was relatively much larger in size. Also its form was not perfectly circular but was



FIGS. 16–20.—Pollen grains germinated in secretion containing 0.5% sulfanilamide. Fig. 16, *E. sinica*, two nuclei organized from two groups of chromosomes and some chromosomes still lying as such. Cf. fig. 22. Figs. 17–19, *E. saxatilis*. Fig. 17, diplochromosomes scattered after spindle inhibition; note chromosome at *S*, bearing a satellite. Cf. fig. 23. Fig. 18, daughter chromosomes separated but still lying parallel. Cf. fig. 24. Fig. 19, single restitution nucleus. Fig. 20, *E. foliata*, seven diplochromosomes scattered owing to spindle inhibition.

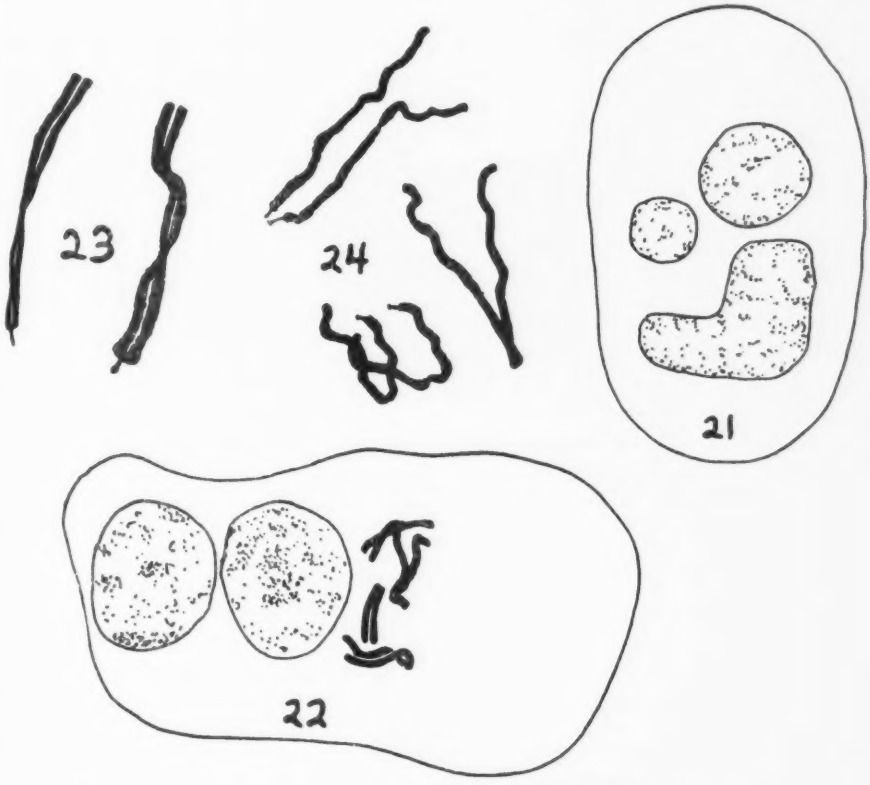
elongated or more or less amoeboid and irregularly lobed. Such a nucleus was less dense and possessed a feebly developed nuclear membrane. In other cases several nuclei of different forms and sizes were organized, some of which were connected with narrow bridge-like processes (figs. 15, 21). In still other cases where chromosomes were widely scattered, a diffuse type of nucleus resulted whose boundary was not differentiated from the cytoplasm. All these cases were equally frequent. The formation of different numbers and different shapes of nuclei was entirely dependent on the method of distribution of daughter chromosomes within the body cell when the process of nuclear formation began. If chromosomes were compactly arranged, a single nucleus was formed; if they were present in two or three groups, each group formed a separate nucleus; and if one or a few chromosomes were present between two groups, a bridge-like process connecting the nuclei was organized in the intermediate region. In case more than one nucleus was organized, it was not necessary for the two daughters of the same parent chromosome to enter the same nucleus, since the daughter chromosomes often had become widely separated. It is obvious, therefore, that the constitution of these different nuclei was highly variable.

It has already been seen that, in some cases, all the metaphase chromosomes but one were grouped together, with the solitary chromosome far apart (fig. 11). In such cases the possibility is that all the chromosomes of the group would form a single nucleus while another would be organized by the two daughters of the separated chromosome. Evidently the larger of such restitution nuclei would be aneuploid, containing the $2n - 2$ number. It is of interest that aneuploid plants have been produced by BLAKESLEE in *Datura* by colchicine treatment (1). DERMEN (2), in reviewing the colchicine effect on plant tissues, suggested that aneuploidy may be due to "partial instead of a total arrest of chromosome division as colchicine begins to become effective and at the time its effect is finally being eliminated" or to "multipolar division in some polyploid nuclei" as observed by LEVAN (5) in his studies on the effect of colchicine on root-tip mitosis in *Allium*. The formation of aneuploid nuclei, in many cases, may also be ascribed to the scattering of chromosomes after the inhibition of the spindle mechanism, since those that wander away may be exluded from entrance into the restitution nucleus on the arrival of the metabolic state.

As a rule, whether one or more nuclei were organized, all the chromosomes within a body cell underwent nuclear organization simultaneously. Occasionally, however, instances were observed where most of the chromosomes had formed a restitution nucleus or nuclei while a few were still in the chromosome stage (figs. 16, 22). Such rare instances were also observed to result from colchicine treatment (6).

The process of nuclear formation in this material was exactly the same as that which occurs during the formation of daughter nuclei from the two telophase groups of chromosomes in a normal division of the body nucleus. The chromosomes in the material treated with sulfanilamide did not, however, always undergo such a high degree of contraction as in the telophase in normal mitosis. The daughter chromosomes became surrounded by some pale substance which contrasted sharply with the dark-gray cytoplasm of the body cell. This pale area in many cases began to develop around daughter chromosomes still closely approximated. This area around each chro-

mosome gradually increased in size, and simultaneously the chromosome lost its staining reaction. It appeared as if some substance was secreted by the chromosome which reacted with the cytoplasm, changing its nature. With the increase in area of this pale material around individual chromosomes, all the chromosomes lying near one another became immersed in a common matrix of this pale substance, while the chromosomes themselves became faded. This entire mass then was transformed into a nucleus. If the chromosomes were closely situated, the matrix was quite compact and the nuclear membrane well developed. On the other hand, if the chromosomes were more or less dispersed, the matrix was relatively thin and the nuclear membrane indistinct. In those cases where the chromosomes were widely scattered, the nuclear substance was in a highly dispersed state, and its boundary was not easily differentiated from the surrounding cytoplasm.



FIGS. 21–24. Cf. figs. 15, 16, 17, and 18, respectively, from which drawings were made

It is clear, therefore, that sulfanilamide in its effective dose has exactly the same effect in bringing about irregularities in mitosis as does colchicine. The form, the number, and the mode of for-

mation of restitution nuclei are the same as after colchicine application, but sulfanilamide must be applied in relatively higher concentration than colchicine to produce equal effect.

Discussion

The results in general agree with those of PETERS (8) and FULLER (4) in the case of *A. cepa* root tips. The present observations, however, do not corroborate PETERS' statement that "cells which were in metaphase or anaphase at the onset of treatment with sulfanilamide [0.5%] complete the nuclear division." The immediate effect of the chemical in the present material is to bring about excitation movements in the cytoplasm, thus disturbing its balance. If the cells are at any of the above stages, the fragile spindle immediately breaks down, and the chromosomes are released. They may become widely dispersed.

No X-shaped diplochromosomes with undivided kinetochore were observed in the present material. Always the chromatids lay parallel. The separation started at the ends until the kinetochore was involved. For a time the daughter chromosomes lay parallel and then lost this arrangement. These results agree with PETERS' observation with sulfanilamide. The effect of colchicine on *Ephedra* pollen grains is the same (6), although PETERS stated that in *A. cepa* the effect of colchicine is different from that of sulfanilamide and that X-shaped diplochromosomes were commonly observed.

Considerable shortening of chromosomes was observed in some pollen grains under the effect of 0.5% sulfanilamide. Such shortening was also observed by PETERS and by FULLER in *A. cepa*.

FULLER stated that a few cells exhibited normal mitosis even in root tips immersed in sulfanilamide for 96 hours. In none of the pollen grains treated with 0.5% sulfanilamide at different mitotic phases or germinated in 0.5% sulfanilamide medium was continuation of normal mitosis observed.

Summary

1. The effect of sulfanilamide has been studied on the division mechanism of the body nucleus in the pollen grains of four species of *Ephedra*. The grains were germinated in artificial culture on the natural mucilaginous material which is secreted by the ripe ovules at their micropylar ends.

2. When sulfanilamide was added to make a concentration of 0.5% in such cultures in which germinating grains exhibited metaphase, anaphase, or telophase stages in the body cell, the immediate effect was the complete collapse of the spindle mechanism.

3. A 0.2% concentration of sulfanilamide in the mucilaginous secretion on which the grains were germinated has no effect on the mitotic phenomena. A 0.4% concentration was just at the critical level for obtaining a paralyzing effect on the spindle mechanism and occasionally brought about the liberation of a chromosome from the spindle zone. In a 0.5% dose the spindle mechanism was completely inhibited, and the chromosomes became scattered, split, and underwent the process of nuclear formation in exactly the same manner as upon treatment with colchicine.

4. The direct effects of the chemical on chromosomes are (*a*) to bring into prominence their kinetochore region and (*b*) to cause in some instances a high degree of contraction. The indirect effect on the chromosomes is that they become straightened because of the absence of a

spindle mechanism. Sulfanilamide, thus, in its effective dose, produces the same effects as colchicine. The former, however, is potent only in higher concentrations, while the latter is effective in relatively much more dilute strengths.

DEPARTMENT OF BOTANY
E. PANJAB UNIVERSITY, AMRITSAR, INDIA

LITERATURE CITED

1. BLAKESLEE, A. F. The present and potential service of chemistry to plant breeding. Amer. Jour. Bot. **26**:163–172. 1939.
2. DERMEN, H. Colchicine polyploidy and technique. Bot. Rev. **6**:599–653. 1940.
3. EIGSTI, O. J. A comparative study of the effect of sulfanilamide and colchicine upon mitosis of the generative cell in the pollen tube of *Tradescantia occidentalis* (Britton) Smyth. Genetics **27**:141–142. 1942.
4. FULLER, T. C. Effect of several sulfa- compounds on nuclear and cell division. BOT. GAZ. **109**:177–183. 1947.
5. LEVAN, A. The effect of colchicine on root mitosis in *Allium*. Hereditas **24**:471–486. 1938.
6. MEHRA, P. N. Colchicine effect on the mitotic division of the body nucleus in the pollen grains of some *Ephedra* species. Nat. Inst. Sci. India **12**:333–340. 1946.
7. ———. A study of the karyotypes and the occurrence of diploid male gametophytes in some species of the genus *Ephedra*. Proc. Nat. Acad. Sci. India **16**:259–286. 1947.
8. PETERS, J. J. Cytological effects of sulfanilamide on *Allium cepa*. BOT. GAZ. **107**:390–392. 1946.
9. TRAUB, H. P. Effect of sulfanilamide and other sulfa- compounds on nuclear conditions in plants. Jour. Hered. **32**:157–159. 1941.

INCORPORATION OF ISOTOPIC CARBON INTO COMPOUNDS BY BIOSYNTHESIS[1]

R. H. BURRIS, P. W. WILSON, AND R. E. STUTZ

Introduction

In the past few years we have had numerous inquiries about techniques for biosynthesis with higher plants, inquiries apparently reflecting a surprisingly widespread unfamiliarity with methods for growing plants in closed systems. The necessity for maintaining bacteriological control and for altering the gaseous atmosphere supplied plants in studies of biological nitrogen fixation first led us to develop simple closed systems for growing plants (**3, 9, 10**). Although we have used these extensively for many years, their details remain unpublished. No attempt is made here to review other described methods for culture of plants in closed systems (**1, 5, 6**) or to consider the insertion of labels other than carbon.

Basic methods

After many trials with different types of containers (**3, 9, 10, 11**) the apparatus finally adopted for most work is that shown in figure 1. A fixed weight or volume of sand is placed in a 9-liter Pyrex serum bottle, and a complete nutrient solution is added to near saturation of the sand. In the nitrogen-fixation studies Crone's nitrogen-free solution is preferred: 0.67 gm. KCl and 0.17 gm. each of $CaSO_4 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$,

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies were supported in part by funds from the Rockefeller Foundation and by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. R. H. BURRIS performed preliminary tests on techniques for biosynthesis by higher plants while at the Argonne National Laboratory during the summer of 1946.

$Ca_3(PO_4)_2$, $FePO_4$, and K_3PO_4 per liter. Other solutions may be substituted; Hoagland's (**2**) has the advantage that all components are soluble in the concentrations used. The bottles are plugged with cotton, capped with paper, and sterilized by heating for 2 hours at 121° C. in an autoclave.

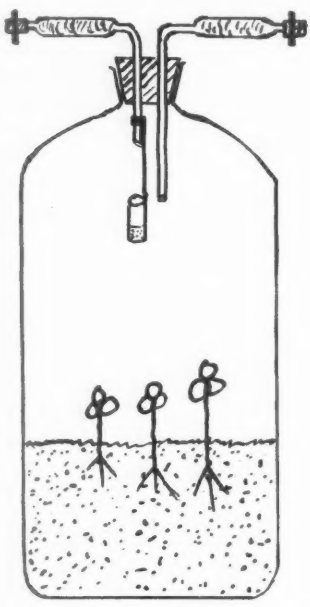


FIG. 1.—Serum bottle equipped for aseptic culture of plants in closed system.

In studies of nitrogen fixation sterilization of seed is mainly concerned with the destruction of the rhizobia (**3**). This can be accomplished with seeds of the common legumes by treatment with the following disinfectants: 70% ethyl alcohol for 3 minutes, three rinses with sterile distilled water, 5–10 minutes with 0.1% mercuric chloride, three rinses, and, finally, 1–3 minutes with calcium or sodium hypochlorite solution containing 3% available chlorine, followed by five rinses. Penetration of each of the disinfectants is improved by placing the seed in a sterile Petri dish kept in a container on which a vacuum is alternately pulled and released. The bacteria-free seeds are distributed on moist blotting paper in sterile Petri dishes and are allowed to germinate in a canister containing water to maintain a saturated atmosphere. The seedlings (e.g., twenty of red clover) are transferred aseptically to the 9-liter serum bottle, and the sand surface is broken and pushed over them with a long metal rod sterilized by dipping in alcohol and flaming. When the plants are 2 inches tall, the paper caps and cotton-plugs are discarded and replaced by sterile rubber stoppers carrying cotton-plugged tubes for adding or withdrawing gases. At weekly intervals the gases are aerated from the bottles and new gas mixtures are added.

In the system described, red clover plants will grow to 12–15 inches without addition of water or nutrients other than the supply initially present. Only CO_2 is added to the bottles; periodically the liberated O_2 is vented. With care the plants can be kept aseptic, although strict asepsis is not always necessary. Insect or microbial damage seldom occurs, and algae do not grow on the surface of the sand.

The necessity for the addition of CO_2 is indicated by the solution contained in a small shell vial suspended from one of the inlet tubes (**7**). WILSON (**8**) described several suitable indicators, but an approximately 0.001 *M* solution of $NaHCO_3$ plus cresol red is commonly used (Clark and Lubs indicator—0.1 gm. cresol red plus 26.2 ml. 0.01 *N* NaOH ground together and diluted to 250 ml. with water; one part of this solution plus 19 parts of 0.001 *M* $NaHCO_3$). When the indicator becomes red, CO_2 is added, and the indicator turns yellow. Carbon diox-

ide is generated and added conveniently from the system shown in figure 2. This figure also shows in outline two alternative systems that permit more complete recovery of the CO_2 generated. These generators are suitable for use with normal carbonates or with radioactive carbonates of low specific activity. When tank CO_2 is employed, the generating apparatus is dispensed with and sections *A*, *B*, and *C* only of figure 2 suffice.

The temperature in the interior of the closed bottles rises substantially above that of the greenhouse on warm, bright days. The bottles can be cooled by spraying cold water through a collar of perforated copper tubing placed around their necks.

Modifications

The procedures developed by WILSON and his associates (**10**) and summarized in the foregoing furnish a basic method for culture of plants in closed systems. A few modifications have been made for biosynthesis of C^{14}- or C^{13}-labeled compounds. Since the small necks of the 9-liter serum bottles (fig. 1) make the removal of large plants difficult, wide-mouthed jars usually have been substituted. The maintenance of aseptic conditions is often unnecessary, but if plants must be exposed to $C^{14}O_2$ for several days or weeks in a closed system saturated with water vapor, fungal growth on the plants may become serious in a nonaseptic environment.

Figures 3–6 illustrate various chambers which we have tested. A plain cylindrical Pyrex jar with a ground open end, obtainable in various sizes, makes a suitable container. Figure 3 shows one closed with a glass plate which is waxed onto the top. A hole drilled in the glass plate is closed by waxing a rubber stopper into place. The tube through the stopper furnishes a port for adding gas and supports the indicator vial. A potted plant may be lowered into the jar and the system sealed for exposure to $C^{14}O_2$.

Figure 4 is a modification in which the

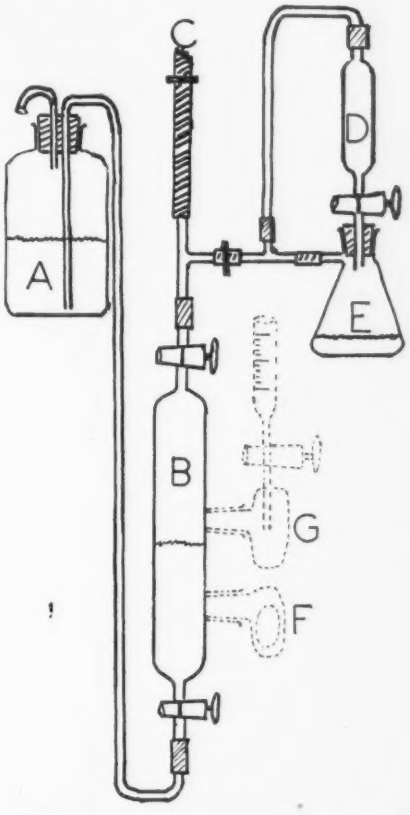
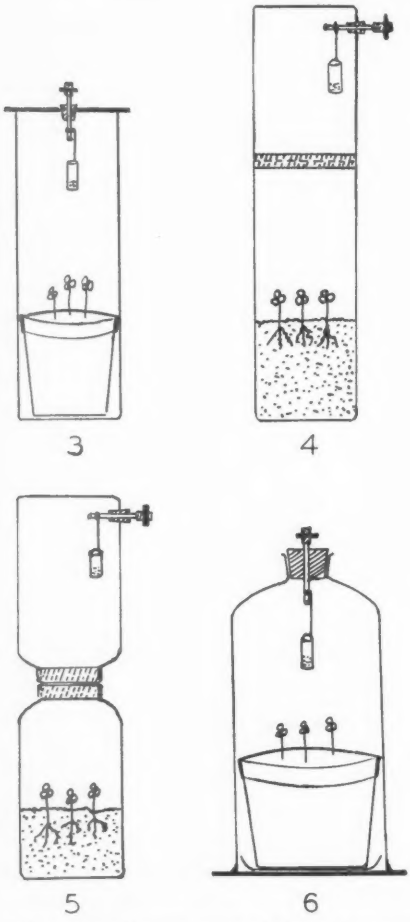


FIG. 2.—Apparatus for generating CO_2 from $BaCO_3$ and for adding CO_2 to plants. Acid (20% HCl or 30% $HClO_4$) is dropped from *D* onto $BaCO_3$ in *E*; the CO_2 generated displaces liquid from *B* into *A*. CO_2 can be added to plants through rubber tubing *C* by displacing the gas from *B* with liquid from *A*. By eliminating sections *D*, *E*, and their connections and substituting outlined section *F* or *G* for generating CO_2, more complete recovery of the CO_2 is achieved. *F* consists of a double-sac sidearm, as illustrated in fig. 8, which can be rotated to mix acid and $BaCO_3$. *G* consists of a well for $BaCO_3$ and a graduated dropping funnel from which a measured amount of acid can be added at intervals; pressure must be used to force acid from dropping funnel.

plants are grown directly in a glass jar, and then another glass jar is sealed over the first at the time of treatment. Potted plants may be dislodged from the pot and carefully transferred with their soil to such a glass jar. The two glass jars are connected and effectively sealed by a 1-inch band of cloth adhesive tape painted with a hot beeswax-rosin mixture (equal parts beeswax and rosin). If an unusually tall container is desired, a length of 6-inch-diameter tubing can be sealed between the bottom jar and the top jar.



FIGS. 3–6.—Various types of closed glass containers for growing plants.

In the arrangement shown in figure 5 the plants are grown in a 2-quart glass canning jar. Two open-top metal jar-cover rings are soldered together, and the two jars are connected by screwing them into the jar rings. The junction is painted with beeswax-rosin. This device, suitable for inclosing small plants for treatment, is inexpensive and simple.[2]

A favorite with many, the bell jar covering a potted plant (fig. 6) is not recommended because of the difficulty of maintaining a good seal. Even though the pot is placed in a saucer, soil frequently gets onto the ground-glass surface of the bell jar or the ground-glass bottom plate. Transpired water also collects at the bottom seal.

A system that has many advantages where it is applicable is shown in figure 7. The plants are grown nonaseptically in porous tapered pots under ordinary greenhouse conditions. Porous pots are preferred to glass containers, as aeration is better and overwatering is less serious. The pot of plants to be treated is lowered into a glass jar, and another jar is sealed over it. The $BaC^{14}O_3$ is weighed into a small test tube through which a hole has been blown in the side near the bottom. The tube is closed at the top with a serum-vial rubber stopper and is sealed into the upper glass jar with beeswax-rosin. If desired, a small vial of indicator solution can be attached to this CO_2-generating tube. Carbon dioxide is generated in the sealed system by injecting 30% perchloric acid through the serum-vial stopper with a hypodermic needle and syringe. It is advisable to moisten the $BaCO_3$ before sealing the vial in place,

[2] Chambers of this same type have been described by J. W. Brown, BOT. GAZ. 107:332–343. 1946.

for otherwise $BaCO_3$ dust may be blown from the tube into the main chamber. Generating CO_2 internally eliminates handling small quantities of the radioactive gas. If the plant photosynthesizes for a period immediately before harvesting, CO_2 is virtually exhausted from the system, so that little hazard from radioactive gas remains when the jars are opened. They should always be opened in a well-ventilated hood, however, and the usual precautions for handling radioactive materials should be observed.

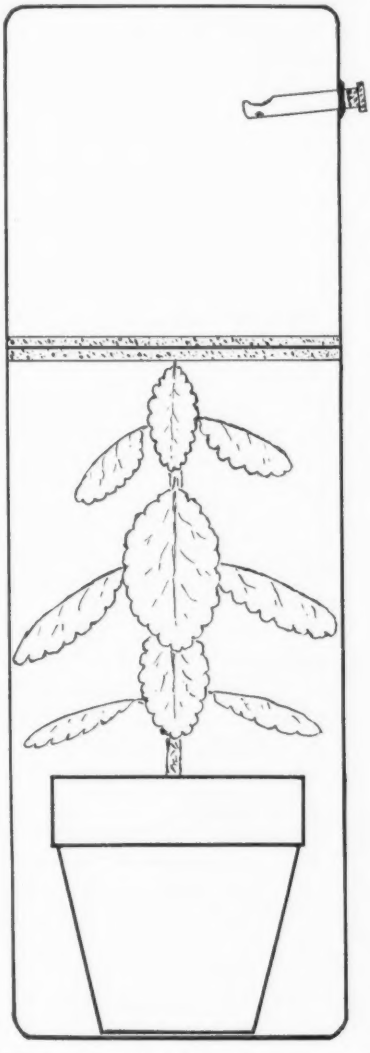


FIG. 7.—Closed plant-culture chamber equipped for internal generation of CO_2.

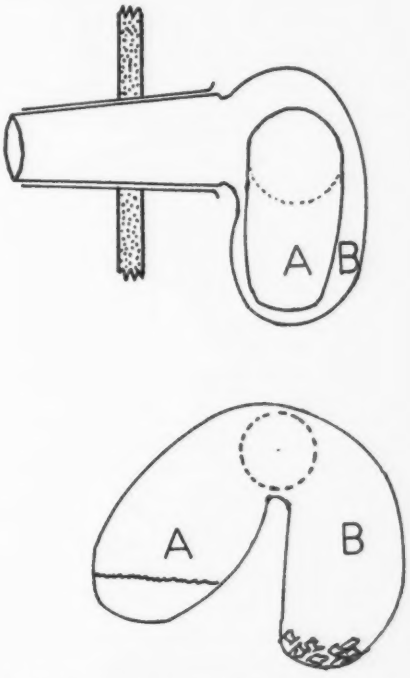


FIG. 8.—Double side sac for generating CO_2. Top diagram shows double side sac on inner joint and standard taper outer joint which can be waxed or sealed into wall of glass container; bottom diagram (shifted 90° from top diagram) shows how acid in side sac *A* can be dumped into $BaCO_3$ in side sac *B* by rotating inner ground-glass joint.

If several additions of CO_2 to the plant are wanted, the CO_2-generating tube is removed, recharged, and replaced. Alternatively, an outer ground-glass joint can be sealed into the jar, and an inner joint carrying a double sac (fig. 8) can be charged with $BaC^{14}O_3$ (sac B) and 30% perchloric acid (sac A). When the joint is rotated, the acid dumps from A to B

and generates CO_2. By dropping small amounts of standard acid through a stopcock (or injecting it through a rubber barrier) onto a charge of $BaC^{14}O_3$ inside the chamber, it would be possible to generate CO_2 in known quantities at intervals. If a circulation of CO_2 more rapid than will occur by diffusion and convection is needed, a small steel fan rotated from the outside with a magnetic stirrer can be placed inside the jar; or a small electric fan with sealed-in leads can be used.

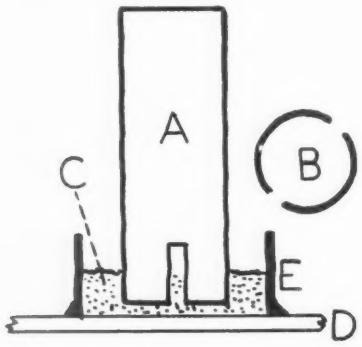


FIG. 9.—Device for grinding holes in glass. Brass tube, *A* (bottom end view, *B*), is connected to drill press and rotates in water suspension of abrasive, *C*, against glass, *D*. Short section of tubing, *E*, is waxed to *D* to form a well to retain abrasive suspension.

Holes are readily drilled in the glass jars by the method described in Corning's booklet, *Laboratory Glass Blowing with Pyrex Glasses*. The holes are drilled with a slotted brass tube (fig. 9), which is rotated in a drill press. It rotates in a mixture of abrasive and water; this is retained by a short length of pipe of larger diameter waxed to the glass surface.

Discussion

The methods described for culture of plants in closed systems are simple and require inexpensive apparatus, yet are satisfactory for biosynthesis of C^{14}- or C^{13}-labeled compounds by higher plants. Although no attempt is made here to recommend particular plants for a given synthesis or to describe methods for separation of labeled compounds, it should be noted that the methods described have been tested in the preparation of labeled starch from bean leaves, labeled isocitric and malic acids from *Bryophyllum calycinum*, and malic and citric acids from tobacco. Partition chromatography on silica gel (**4**) has been our method of choice for separation of organic acids, but other accepted methods can be readily adapted to the recovery of the desired compounds. Neither do we wish to infer that all plants can be grown readily from germination to maturity in closed systems—some will undoubtedly give difficulties. Although a comparatively short period of treatment with $C^{14}O_2$ is usually sufficient to label wanted compounds, materials that are formed at a relatively slow rate may require prolonged exposure of the plant to $C^{14}O_2$ of low specific activity. Excised leaves from large plants will often serve as well as the intact plant for short exposures.

Large, bench-sized closed systems for biosynthesis have been proposed, but there is an inherent danger that changes of pressure accompanying daily temperature fluctuations will cause leakage of radioactive gases. It may be argued that such chambers are designed for use with diluted $C^{14}O_2$ and for production of large quantities of plants of low radioactivity. The opposite point of view—that materials should be produced with high specific activity in small, easily controlled chambers and then diluted with normal plant products—should be carefully considered, however, for it has much to recommend it. It may be pointed out also that two small tobacco plants (about 14 inches high), which we sealed into a glass jar and checked recently under winter

greenhouse conditions, accumulated 1100 ml. of normal CO_2 in 3 days. This is equivalent to about 85 millicuries of $C^{14}O_2$ at the concentration currently supplied from Oak Ridge and corresponds to a yearly consumption of about 10,000 millicuries or $500,000 worth of C^{14}. There is a possibility of radiation damage to the plant at too high concentrations of $C^{14}O_2$, but this is probably not serious. Our budget has not permitted a critical test of the point.

Summary

With simple equipment it is possible to biosynthesize C^{14}- or C^{13}-labeled compounds by growing plants in closed systems. Several suitable containers for plants and devices for generating and adding carbon dioxide are described.

DEPARTMENTS OF BIOCHEMISTRY AND AGRICULTURAL BACTERIOLOGY
COLLEGE OF AGRICULTURE
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. GEILING, E. M. K.; KELSEY, F. E.; MCINTOSH, B. J.; and GANZ, A. Biosynthesis of radioactive drugs using carbon 14. Science **108**:558–559. 1948.
2. HOAGLAND, D. R., and ARNON, D. I. The water-culture method for growing plants without soil. Univ. Calif. Agr. Exp. Sta. Circ. **347**. 1938.
3. HOPKINS, E. W.; WILSON, P. W.; and FRED, E. B. A method for the growth of leguminous plants under bacteriologically controlled conditions. Jour. Amer. Soc. Agron. **23**:32–40. 1931.
4. ISHERWOOD, F. A. The determination and isolation of organic acids in fruit. Biochem. Jour. **40**: 688–695. 1946.
5. LIVINGSTON, L. G., and MEDES, GRACE. The biosynthesis of C^{13} compounds. I. The biosynthesis of C^{13}-labeled starch. Jour. Gen. Physiol. **31**:75–88. 1947.
6. PUTMAN, E. W.; HASSID, W. Z.; KROTKOV, G.; and BARKER, H. A. Preparation of radioactive carbon-labeled sugars by photosynthesis. Jour. Biol. Chem. **173**:785–795. 1948.
7. SMYTH, ELIZABETH M. The determination of CO_2 content of an atmosphere in a closed system. Science **80**:294. 1934.
8. WILSON, P. W. Colorimetric method for determination of CO_2 in gas mixtures. Science **78**: 462–463. 1933.
9. ———. Mechanism of symbiotic nitrogen fixation. I. The influence of pN_2. Jour. Amer. Chem. Soc. **58**:*1256–1261. 1936.*
10. ———. The Biochemistry of Symbiotic Nitrogen Fixation. Univ. Wisconsin Press, Madison. 1940.
11. WILSON, P. W., and GEORGI, C. E. Methods for controlling the environment of greenhouse plants. BOT. GAZ. **94**:346–363. 1932.

EFFECTS OF SEVERAL PLANT GROWTH-REGULATORS ON WOUND HEALING OF SUGAR MAPLE[1]

EDWIN A. DAVIS[2]

Introduction

The concept of wound hormones from the time of its formulation to the present day has remained popular in many botanical centers. It would seem worth while to trace briefly the development of the wound-hormone theory.

The concept of a wound substance was formulated by WIESNER (*18*), who proposed that a substance flows out of wounded cells which causes the adjoining uninjured cells to become meristematic, resulting in the formation of a callus. MASSART (*11*) later observed cell divi-

[1] This work was supported by a fellowship from the F. A. Bartlett Tree Expert Company, Stamford, Connecticut.

[2] Present address: Carnegie Institution of Washington, Div. of Plant Biol., Stanford, Calif.

sion in the area surrounding dying or necrotic cells and thought that there was a causal connection, probably of a chemical nature. HABERLANDT (7) dealt with the wound-hormone theory experimentally. From his experiments in which he compared the number of cell divisions on the surface of washed and unwashed Kohlrabi tuber slices, and on the surface of slices which were covered with a thin layer of Kohlrabi tissue pulp, he concluded that he had demonstrated the wound hormone by the effectiveness of the decomposition products from the dying cells in inducing cell division. NAKANO (12), in order to explain the observation that parenchyma cells located between a vascular bundle and the wound surface divide more vigorously than those not so situated, postulated the combined action of an irritant (wound hormone) diffusing from the wound surface and a factor from the vascular bundle (leptohormone). REICHE (15) injected tissue juices and cell debris into the intercellular systems of stems and petioles of different plants and found that the resting cells bordering the intercellular spaces were stimulated to divide. He considered that, when a plant organ is wounded, wound-stimulating materials arise which can be conducted away by diffusion in living cells, intercellular spaces, and in the lumen of vessels, or mechanically by capillarity. PETRIE (14) studied cork formation in artificial wounds of leaves of *Echeveria secunda* and of potato tubers. He believed the wound hormone of HABERLANDT to be an oxidation product of a compound, contained in living cells, which is normally inactive but which can be made active by atmospheric oxygen or oxidases. He did not think the wound substance should be classified as a hormone analogous to animal hormones.

BROWN (2) investigated the response of the vascular cambium of leader shoots of the balsam poplar (*Populus balsamifera* L.) to wounding. The investigation was carried out in the laboratory during the winter months, at which time it was possible to make observations on local cambial activity distinct from normal cambial activity. It was found that, the greater the amount of bark distal to a wound, the greater was the local cambial activity in relation to the wound. Developing buds and leaves were also found to stimulate this process. Cambial activity in relation to wounding was similar to normal cambial activity in its response to gravity; that is, it was greater on the upper side of a horizontally placed shoot than on the lower side. From these results BROWN suggested that a hormone, present in the living bark and also produced by developing buds and leaves, plays a part in the wound reaction. He considered it probable that it is the same hormone involved in normal cambial activity. The observation that from the lower edge of a complete ring a very weak cambial activity developed, the cells from which did not differentiate into vessels and fibers, suggested that a wound substance capable of promoting cell division only was involved. The amount of this wound substance produced seemed to be proportional to the extent of dying cells in the bark subsequent to wounding. Local cambial stimulation above a complete ring involved both cell division and differentiation and was interpreted as the result of both the wound substance and the cambial hormone in the bark traveling in a basipetal direction.

More recently the study of the wound reaction has involved the use of plant growth-regulators. BROWN and CORMACK (3) reported that heteroauxin in lanolin, applied to the distal end of disbudded

cuttings of *P. balsamifera* L., stimulated cambial activity for a distance of 1–1.5 inches below the point of application. Cambial activity was also stimulated in a bridged ring considerably below the point of application but was absent between the area of stimulation at the point of application and the bridged wound. The wood which was laid down at and immediately below the point of application was found to be abnormal, containing numerous nests of parenchyma and vessels which were in general narrower than normal. JAKEŠ and HEXNEROVÁ (9) noted that 1% indoleacetic acid in lanolin and "Bevitan" paste were effective in the wound treatment of fruit trees. GOUWENTAK and MAAS (6), working with disbudded shoots of *Fraxinus ornus*, *Populus nigra*, and *Salix fragilis*, found that, when freshly cut surfaces at the upper ends were treated with lanolin containing heteroauxin, large amounts of wood were produced along the entire length of the shoots.

Investigations have also been carried out in which compounds thought to be at least in part responsible for the wound reaction have been determined. ORSÓS (13) found that the so-called "wound reaction" (i.e., enzymatic activity, cell division, and cell growth) could be produced in Kohlrabi with the aid of protein decomposition products. Through the action of these products the hydrogen-ion concentration within the cells increased. Tyrosine was found to be released in the wound reaction and was considered to be the effective component of the decomposition products. ENGLISH *et al.* (4) isolated a crystalline substance from stringbean pods which is capable of inducing renewed cell division and extension in the parenchymatous cells of the bean pod mesocarp. The structure of the compound was determined and was synthesized (5); the compound was termed "traumatic acid" and was considered by them to be a plant wound hormone. HAMMETT and CHAPMAN (8) found a correlation between the sulfhydryl group, mitosis, and cell elongation in roots of *Phaseolus vulgaris*. The highest concentration of —SH was found to be in the region of highest proliferation and the lowest concentration of —SH in the region of cell elongation. Upon making a scratch along the length of a root, a strong —SH reaction was almost immediately produced along the line of injury. The —SH reaction was also intensified by crushing. These observations indicate that —SH is liberated by trauma.

From a practical standpoint the ideal wound dressing has yet to be discovered. TILFORD (16) found that lanolin containing indolebutyric acid did not stimulate callus formation and indicated that a greater effort should be made to stimulate healing by incorporating growth substances in wound dressings.

The purpose of this paper is to report the results of an experiment in which several plant growth-regulating substances were tested for their abilities to stimulate the wound healing of sugar maple. It would be of considerable theoretical as well as practical interest if any of the compounds tested were able to act in the capacity of a wound substance.

Material and methods

The experimental tree was the sugar maple, *Acer saccharum* Marsh. A total of 120 trees, located on the grounds of the Bartlett Tree Research Laboratories at Stamford, Connecticut, were used. They ranged from approximately 2 to 6 inches in diameter and were arranged in an experimental plot which was divided into four replications, each containing thirty

trees. A view of some of the trees is shown in figure 1.

Six compounds were tested for their abilities to stimulate wound healing: 3-indolebutyric acid, *o*-chlorophenoxypropionic acid, *p*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, traumatic acid, and glutathione.[3]

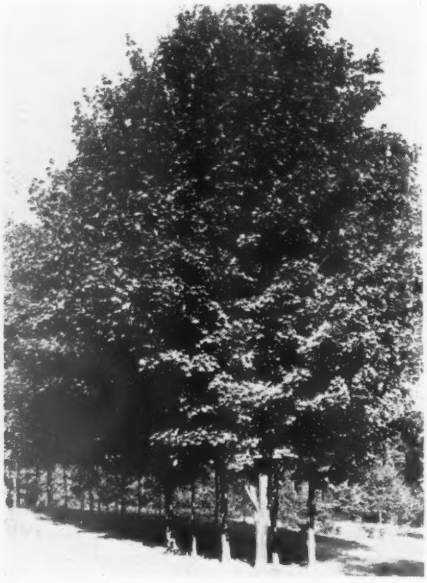


FIG. 1.—View of some of sugar maple trees after wounds were made, treated, and covered with adhesive tape.

Glutathione is the tripeptide of glutamic acid, glycine, and cysteine, the latter being a source of the sulfhydryl group. Talc was used as the carrier. The compounds were prepared in concentrations of 200, 100, 50, 1.0, and 0.1 mg/gm talc. The preparations of *o*-chlorophenoxypropionic acid, 2,4-dichlorophenoxyacetic acid, traumatic acid, and *p*-chlorophenoxyacetic acid were prepared by making solutions at the appropriate concentrations in 50% ethyl alcohol so that, when 1 ml. of solution was added to a gram of talc, the desired concentration of the compound was achieved. The preparations were thoroughly mixed, and the alcohol evaporated off. Then the preparations were again mixed in order to insure uniform distribution of the compounds throughout the talc. The preparations of 3-indolebutyric acid and glutathione were prepared in a similar manner except that the chemicals were dissolved in 95% ethyl alcohol and distilled water, respectively. The control preparation consisted of talc. There was a total of thirty treatments which were assigned to the trees in each replication at random.

The trunk of each tree contained four wounds, three of which were treatment wounds, the other a control. All the treatments on any given tree were the same. The wounds were arranged along the longitudinal axes of the trees approximately 1 foot apart, each wound facing a different direction. The top wound faced north, the second east, the third south, and the fourth west. The controls in each replication faced in different directions and were in different positions along the trunks of the trees in relation to the other wounds. In the first replication the top wounds were the controls and faced north; in the second, the second wounds from the top were the controls and faced east; in the third, the third wounds from the top were the controls and faced south; and in the fourth replication the bottom wounds were the controls and faced west. The treatment

[3] I wish to thank Dr. JAMES ENGLISH, JR., for the sample of traumatic acid, the Dow Chemical Company for samples of 3-indolebutyric acid, *p*-chlorophenoxyacetic acid, *o*-chlorophenoxypropionic acid, and 2,4-dichlorophenoxyacetic acid. I also thank the Dupont de Nemours and Company for a sample of 2,4-dichlorophenoxyacetic acid.

wounds were assigned to the other positions.

The wounds were rectangular in shape with an area of 22.2 cm.^{2} and were made with a specially designed tool. The bark was cut and removed in one piece. The surface of the wound was thoroughly scraped to remove all the phloem elements or cambial cells which may have adhered to the wood.

The amount of talcum preparation for each wound was measured with a standard measure which held approximately 0.1 gm. The preparations were placed in the cups of color reaction plates and were dabbed on the wounds with small wads of absorbent cotton immediately after the wounds were made. A fresh wad of cotton was used for each preparation. The wounds were completely covered, making particularly certain that the edges of the bark and the regions where the bark and wood came in contact were well coated. The wounds were covered with surgical adhesive tape. There was a total of 480 wounds.

The wounds were made and treated between July 29 and August 8, 1946. At this time the spring surge of cambial activity had ceased, and the slower process of midsummer growth had commenced. Readings were made the following May. In order to judge the amounts of healing, outlines of the areas of the wounds not healed over were traced on strips of celluloid. The outlines were then transferred to paper where their areas were measured with a planimeter. These areas were subtracted from the initial wound area, giving the amount of healing-over in square centimeters. For each tree the average amount of healing for the three treatment wounds was calculated. The results are expressed as the differences between the amounts of healing of treatment and of control wounds.

Results

The results are compiled in table 1. Plus values indicate that healing was stimulated and negative values that it was inhibited.

3-Indolebutyric acid did not stimulate callus formation or the subsequent processes of healing at any of the concentrations tested. In general, the healing of the control wounds was superior to the healing of the treatment wounds. Although this compound retarded the wound-healing process, it did not cause a noticeable dieback of the surrounding tissues.

o-Chlorophenoxypropionic acid did not stimulate healing at the concentrations tested. As a rule the healing of the treatment wounds was poorer than the healing of the controls. The concentration 1 mg/gm was found to be somewhat better than the other concentrations. The differences, however, were not great enough to be significant.

p-Chlorophenoxyacetic acid was also ineffective in stimulating healing. In most cases the control wounds demonstrated a greater amount of healing than the treatment wounds.

2,4-Dichlorophenoxyacetic acid was inhibitory at the four highest concentrations. At 200 mg/gm it caused a considerable degree of dieback. Concentrations of 100, 50, and 1.0 mg/gm were also inhibitory, but to a lesser extent. From 1.0 to 200 mg/gm the amount of dieback was proportional to the concentration of the compound applied. At 0.1 mg/gm there was some indication of stimulation. It was not great enough, however, to be significant. It is possible that by using a larger number of pairs this treatment could be shown to be beneficial.

Traumatic acid was not found to accelerate the wound-healing process. In the majority of cases the healing of the control wounds was more rapid than the

TABLE 1

WOUND HEALING OF SUGAR MAPLE AS AFFECTED BY SEVERAL PLANT GROWTH-REGULATING SUBSTANCES. AREAS IN SQ. CM.

TEST COMPOUND	CONCENTRATION (MG/GM TALC)	AREA HEALED OVER			TEST COMPOUND	CONCENTRATION (MG/GM TALC)	AREA HEALED OVER		
		Treatment	Control	Difference			Treatment	Control	Difference
3-Indolebutyric acid	200	5.8	3.9	+1.9	*p*-Chlorophenoxyacetic acid	200	0	4.0	−4.0
		7.2	9.0	−1.8			1.5	2.2	−0.7
		4.6	4.9	−0.3			3.5	7.8	−4.3
		5.4	6.6	−1.2			0	2.8	−2.8
	100	7.1	8.9	−1.8		100	4.0	8.7	−4.7
		5.4	7.7	−2.3			6.1	8.4	−2.3
		5.6	8.7	−3.1			3.6	5.6	−2.0
		4.7	5.6	−0.9			5.0	4.9	+0.1
	50	6.9	7.0	−0.1		50	0.8	7.2	−6.4
		3.4	8.9	−5.5			3.5	5.4	−1.9
		6.2	5.7	+0.5			5.0	4.8	+0.2
		1.8	4.1	−2.3			5.6	6.8	−1.2
	1.0	4.2	0.4	+3.8		1.0	6.3	8.6	−2.3
		2.9	8.2	−5.3			4.3	8.3	−4.0
		8.2	11.7	−3.5			5.1	5.3	−0.2
		8.2	7.8	+0.4			7.0	7.6	−0.6
	0.1	0.8	2.8	−2.0		0.1	3.2	1.7	+1.5
		1.0	4.6	−3.6			3.6	5.4	−1.8
		4.6	5.1	−0.5			6.4	3.1	+3.3
		7.3	8.0	−0.7			6.7	6.9	−0.2
o-Chlorophenoxypropionic acid	200	0.5	3.2	−2.7	2,4-Dichlorophenoxyacetic acid	200	0	2.4	−2.4
		2.8	6.0	−3.2			0.4	5.3	−4.9
		3.5	7.8	−4.3			0	1.7	−1.7
		4.5	6.2	−1.7			0	4.2	−4.2
	100	3.5	0	+3.5		100	0	9.2	−9.2
		3.9	8.3	−4.4			1.0	5.6	−4.6
		2.2	6.3	−4.1			0.4	9.4	−9.0
		1.7	6.5	−4.8			2.5	6.5	−4.0
	50	0	0.4	−0.4		50	0.4	3.8	−3.4
		2.2	5.7	−3.5			3.2	7.6	−4.4
		1.9	7.0	−5.1			0	2.8	−2.8
		6.5	5.0	+1.5			0	7.2	−7.2
	1.0	3.9	2.6	+1.3		1.0	1.0	3.0	−2.0
		0	3.2	−3.2			1.0	1.8	−0.8
		7.0	6.2	+0.8			4.2	4.4	−0.2
		5.7	3.9	+1.8			3.5	0	−3.5
	0.1	3.6	3.9	−0.3		0.1	8.2	7.5	+0.7
		6.2	4.2	+2.0			0.6	4.2	−3.6
		6.6	6.7	−0.1			4.6	2.5	+2.1
		0	0	0			3.8	1.3	+2.5
Traumatic acid	200	1.5	3.3	−1.8	Glutathione	200	2.8	0	+2.8
		3.2	0.2	+3.0			3.2	4.7	−1.5
		0.3	1.2	−0.9			4.5	7.0	−2.5
		4.8	5.7	−0.9			4.8	6.5	−1.7
	100	2.2	6.4	−4.2		100	2.8	0	+2.8
		3.9	9.3	−5.4			5.0	5.8	−0.8
		4.3	10.9	−6.6			5.8	2.0	+3.8
		4.1	9.0	−4.9			4.2	3.3	+0.9
	50	4.0	3.4	+0.6		50	3.6	1.4	+2.2
		2.0	5.1	−3.1			0.9	1.1	−0.2
		8.1	7.1	+1.0			2.9	1.0	+1.9
		5.0	6.5	−1.5			4.6	0.9	+3.7
	1.0	4.9	6.8	−1.9		1.0	4.2	3.6	+0.6
		0.6	2.0	−1.4			2.0	4.0	−2.0
		5.8	5.0	+0.8			3.7	4.7	−1.0
		7.3	7.9	−0.6			4.6	0.5	+4.1
	0.1	3.6	4.5	−0.9		0.1	5.8	6.2	−0.4
		5.5	8.9	−3.4			3.4	5.7	−2.3
		7.8	6.2	+1.6			3.6	10.2	−6.6
		3.8	3.4	+0.4			5.4	5.9	−0.5

healing of the treatment wounds. Root formation was observed from the top horizontal portions of two wounds on the same tree treated with traumatic acid at 200 mg/gm. The other ten wounds which received the same treatment did not exhibit root formation. It was undoubtedly therefore a chance occurrence. Roots did not develop from any of the other wounds of the experiment.

Glutathione was not stimulatory at 0.1, 1.0, and 200 mg/gm talc but caused an apparent stimulation at 50 and 100 mg/gm. An analysis of variance (table 2) indicated that the treatment as a whole, including all the concentrations, did not significantly stimulate healing. The concentrations 50 and 100 mg/gm did, however, stimulate wound healing to a significantly greater degree than the concentrations 0.1, 1.0, and 200 mg/gm. The F value 8.38 surpasses the 5% point for significance but falls short of the 1% point for a high degree of significance. There was no indication of a significant difference between the replications. When the data for the concentrations 50 and 100 mg/gm were pooled, the differences between treatments and controls were found to be significant by the t test. A larger number of pairs would be necessary to demonstrate the significance of the individual treatments 50 and 100 mg/gm.

As a result of the shape of the wounds the greatest amount of wood was produced along the top horizontal portions. This was true of the treatment wounds as well as the controls. This phenomenon is of general occurrence. The wood formed from the top horizontal portions of the wounds was usually warty in appearance.

Discussion

The only compound tested which was found to stimulate wound healing of sugar maple was glutathione. The other compounds gave negative results. It is possible that 2,4-dichlorophenoxyacetic acid at extremely low concentrations would stimulate wound healing. It is also possible that more extensive experiments with *o*-chlorophenoxypropionic acid would indicate it to be stimulatory.

It is of interest that traumatic acid did not stimulate wound healing of sugar maple. This compound was isolated from string-bean pods (4) and is capable of inducing cell division and extension in the parenchymatous cells of the mesocarp of such pods. This reaction is completely nonspecific, as an array of organic compounds, as well as sodium chloride and water, will evoke such a response (10, 17). BONNER and ENGLISH (1) recognized the abilities of many compounds to produce this nonspecific response and

TABLE 2

ANALYSIS OF VARIANCE OF AMOUNTS OF HEALING CAUSED BY GLUTATHIONE

Source of variation	D.F.	S.S.	M.S.	F
Total	19	136.03		
Treatment	4	51.95	12.99	3.10
(0.1+200) vs. (50+100)	(1)	35.10	35.10	8.38*
Remainder	(3)	16.85	5.62	1.34
Replicates	3	33.83	11.28	2.69
Error	12	50.25	4.19	
Mean	1			

* Significant: F surpasses the 5% point, 4.75, for 1 and 12 d.f.

explained it on the basis that they liberated a wound hormone within the tissue itself. The fact that a compound stimulates the parenchymatous cells of the string-bean pod to divide is not adequate proof that it is a wound hormone. It is unfortunate that the idea has been conveyed that traumatic acid is a plant wound hormone. If it is at all, it is probably specific for the string bean.

In order to establish a compound as a wound hormone, or probably more correctly as a wound substance, it is necessary to demonstrate that it is produced by injury, not disintegration, and that it stimulates cell increase in number. HAMMETT and CHAPMAN (8) thought that the only compound as yet discovered which fulfils these requirements is one containing the —SH group. It is, therefore, of some theoretical as well as practical interest that glutathione, which contains the sulfhydryl group, was found to stimulate the wound healing of sugar maple. The mechanism by which the —SH group brings about cell increase in number is not known.

Other —SH-containing compounds should be tested for their abilities to stimulate healing. It is possible that some of these could be found to have a greater stimulating effect than glutathione. From a practical standpoint glutathione is probably too expensive to be utilized commercially. There are, however, other —SH-containing compounds which are less expensive, and it is possible that some of these could be of practical importance when incorporated in wound dressings.

Summary

1. Six growth-regulating substances (3-indolebutyric acid, *o*-chlorophenoxypropionic acid, 2,4-dichlorophenoxyacetic acid, *p*-chlorophenoxyacetic acid, traumatic acid, and glutathione) were tested for their abilities to stimulate the wound healing of sugar maple (*Acer saccharum* Marsh).

2. Glutathione at 50 and 100 mg/gm talc was found to have a stimulating effect.

3. The other compounds at the concentrations tested were not found to stimulate healing.

4. It is indicated that —SH-containing compounds might be of practical importance when incorporated in wound dressings.

I am very grateful to Dr. RUSH P. MARSHALL for his aid and suggestions and to the F. A. Bartlett Tree Expert Company for its support and the use of its facilities. I thank CHARLES B. DAVIS for the tools which were used in this investigation.

OSBORN BOTANICAL LABORATORY
YALE UNIVERSITY
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. BONNER, J., and ENGLISH, J., JR. A chemical and physiological study of traumatin, a plant wound hormone. Plant Physiol. **13**:331–348. 1938.
2. BROWN, A. B. Activity of the vascular cambium in relation to wounding in the balsam poplar, *Populus balsamifera* L. Canad. Jour. Res. **15**: 7–31. 1937.
3. BROWN, A. B., and CORMACK, R. G. H. Stimulation of cambium activity, locally in the region of application and at a distance in relation to a wound by means of heteroauxin. Canadian Jour. Res. **15**:433–441. 1937.
4. ENGLISH, J., JR.; BONNER, J.; and HAAGEN-SMIT, A. J. The wound hormones of plants. II. The isolation of a crystalline active substance. Proc. Nat. Acad. Sci. **25**:323–329. 1939.
5. ———; ———; and ———. Structure and synthesis of a plant wound hormone. Science **90**: 329. 1939.

6. GOUWENTAK, C. A., and MAAS, A. L. Kambiumtätigkeit und Wuchsstoff. II. Mededeel. Landbouwhoogesh. Wageningen **44**:3–16. 1940.
7. HABERLANDT, G. Über Zellteilungshormone und ihre Beziehung zur Wundheilung, Befructung, Parthenogenesis, und Adventivembryonie. Biol. Zentr. **42**:145–172. 1922.
8. HAMMETT, F. S., and CHAPMAN, S. S. A correlation between sulfhydryl, mitosis, and cell growth in length in roots of *Phaseolus vulgaris*. Growth **2**:297–302. 1938.
9. JAKEŠ, E., and HEXNEROVÁ, H. Über den Einfluss der Wuchsstoffe auf Wundgewebebildung bei Obstbäumen. Gartenbauwiss. **13**:83–93. 1939.
10. JOST, L. Wuchsstoff und Zellteilung. Ber. deut. bot. Ges. **53**:733–750. 1935.
11. MASSART, J. La Cicatrisation chez les vegetaux. Mém. Cour. Acad. Roy. Sci. Belg. **57**:2–68. 1898.
12. NAKANO, H. Untersuchungen über Kallusbildung und Wundheilung bei Keimpflanzen. II. Ber. deut. botan. Ges. **42**:267–272. 1924.
13. ORSÓS, O. Untersuchungen über die sogenannten Nekrohormone. Protoplasma **26**:351–371. 1936.
14. PETRIE, L. Experienze sulla formazione del sughero delle ferrite. Boll. Staz. Patol. Veg. (Florence) **9**:328–352. 1929.
15. REICHE, H. Über Auslösung von Zellteilungen durch Injektion von Gewebesäften und Zelltrümmern. Zeits. Bot. **16**:241–278. 1924.
16. TILFORD, P. E. Tree wound dressings. Nation. Shade Tree Conf. [U.S.A.] Proc. Ann. Meet. **16**:41–54. 1940.
17. WEHNELT, B. Untersuchungen über das Wundhormon der Pflanzen. Jahrb. wiss. Bot. **66**:773–813. 1927.
18. WIESNER, J. Die Elementarstruktur und das Wachstum der lebenden Substanz. Wien. 1892.

SOME EFFECTS OF THE VAPOR OF 2,4-DICHLOROPHENOXYACETIC ACID DERIVATIVES ON VARIOUS FIELD-CROP AND VEGETABLE SEEDS

WENDELL R. MULLISON AND RICHARD W. HUMMER

Introduction

It has been shown that the lower alkyl esters, 1–5 carbon atoms, of 2,4-dichlorophenoxyacetic acid (2,4-D) possess sufficient volatility for their vapors to affect growing green plants (**2**, **3**). This paper describes investigations carried out to determine if 2,4-dichlorophenoxyacetic acid derivatives were volatile enough to have an effect on dry seeds with respect to viability, rate of germination, and seedling characteristics.

Material and methods

Half-gallon, wide-mouth, glass canning jars with a Kerr-type screw top and lid were used as storage containers for exposing the seeds to the chemicals. An eye bolt was fastened to the lid through a hole in the center and secured with two nuts, one being placed on the top and the other on the bottom of the lid. Sealing wax was placed over and around the top nut to insure that the container would be airtight when the lid was in place. A sufficient amount of the 2,4-D derivative being tested was placed in a $1\frac{3}{4}$-inch Petri dish to cover its bottom, and the dish was then placed in the bottom of the jar. Small cheesecloth bags containing the seeds to be exposed were suspended from the eyelet of the eye bolt, and the lid was put on the jar (fig. 1), with the bags arranged in such a way as to avoid contact with the walls of the jar. After the screw cap had been tightened to make the jar airtight, the container was placed in a constant temperature room at 80° ± 2° F. (26.7 ± 1.1° C.).

Fifty or one hundred seeds of the vegetable or crop being tested were exposed in the space over the chemical for periods of 30, 60, or 180 days. Following this treatment they were germinated in flats

filled with clean, moist sand, and seedling counts were made.

Experimentation

EXPERIMENT I.—The data for seeds exposed for 30 days to various 2,4-D derivatives are given in table 1.

EXPERIMENT II.—To extend the scope of experiment I, the seeds of some other common vegetable and field crops were exposed for 30 days to the isopropyl ester of 2,4-dichlorophenoxyacetic acid and to the isopropyl ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The results are summarized in table 2.

FIG. 1.—Apparatus used for exposing seeds to vapors of 2,4-D derivatives.

EXPERIMENT III.—In experiment I, with a 30-day exposure period, marked depression of germination was noticed with cucumbers, rye, turnips, and peas. An experiment was then set up to test the effect of longer exposure. It was thought that such an experiment might show susceptibility in a greater range of species. The data for seeds exposed for 60 days to various 2,4-D derivatives are given in table 3.

It was thought desirable to determine whether these results would be different after a much longer exposure period. As cucumber seeds are fairly sensitive to 2,4-D, they were exposed for 180 days to various 2,4-D derivatives. The results are given in table 4.

EXPERIMENT IV.—From the results obtained in the previous experiments the question arose as to whether the 2,4-D from the volatile esters was entering the seed or whether it was adhering to the surface of the seed coat. A preliminary experiment gave indications that the 2,4-D derivatives might be adsorbed on the seed coat without entering the embryo. A further experiment was therefore designed to clarify this point and, in addition, to determine whether 2,4-D from the seed coat entered the embryo during the imbibition process in seed germination. Cucumber seeds were exposed to the methyl ester of 2,4-D for 180 days. They were then divided into two groups of fifty, one of which was put on moist filter paper overnight and the other not. Each of these groups was then subdivided into two more, one of which had the seed coats removed and the other not. Thus four treatments of twenty-five seeds each were planted. The results are given in table 5.

EXPERIMENT V.—Vapor-pressure data on the methyl, ethyl, and isopropyl esters of 2,4-D were obtained from boiling-point determinations over a series of pressures below 760 mm. of mercury. Lines of best fit to the experimental data were obtained by the method of least squares. Extrapolation of these lines gave the values recorded in table 6.

The method is subject to sufficient error, so that the small differences between compounds in table 6 are probably not significant. The vapor-pressure data would classify the lower esters of 2,4-D as having a relatively low vapor pressure from the physical standpoint.

Confirmation of the volatility of these esters was obtained by placing weighed amounts in tared Petri dishes in a vacuum desiccator over silica gel. Over the course of a week with repeated evacuations of the desiccator there was appreciable loss of weight from the methyl, ethyl, and isopropyl esters, but there was

TABLE 1

PERCENTAGE OF SEEDS GERMINATING IN 10 DAYS AFTER 30-DAY EXPOSURE TO VAPOR OF VARIOUS 2,4-D DERIVATIVES

SEEDS	UNTREATED CONTROL	DERIVATIVES OF 2,4-DICHLOROPHENOXYACETIC ACID							ISOPROPYL ESTER 2,4,5-T
		Na salt	Alkanolamine salt	Methyl ester	Ethyl ester	Isopropyl ester	n-butyl ester	n-amyl ester	
Tomato	86	77	85	63	74	78	69	83	85
Bean	98	100	94	86	92	96	98	98	92
Corn	96	100	98	96	98	92	94	100	98
Cucumber	64	72	70	14	16	40	38	68	58
Rye	44	40	45	14	10	14	26	30	15
Wheat	70	68	67	71	75	67	69	65	63
Turnip	80	86	88	12	2	30	22	44	81
Cotton	37	37	40	33	20	28	25	31	40
Pea	96	96	94	32	14	40	90	98	88

TABLE 2

PERCENTAGE OF SEEDS GERMINATING IN 10 DAYS AFTER 30-DAY EXPOSURE TO VAPOR FROM ISOPROPYL ESTERS OF 2,4-D AND 2,4,5-T

Seeds	Untreated control	Isopropyl 2,4-dichlorophenoxyacetate	Isopropyl 2,4,5-trichlorophenoxyacetate
Lima bean	68	44	58
Soy bean	76	66	80
Spinach	23	0	9
Watermelon	92	70	94
Sugar beet	73	20	56
Peanut*	36	18	12
Onion*	70	27	65
Eggplant*	53	21	39

* Seeds in this group are a little slower in their germination; therefore 15-day counts are recorded for them.

no detectable loss from the purified triethanolamine salt or from the free acid of 2,4-D.

Discussion

An experiment with a 15-day exposure period was first performed. This experiment was not replicated. No statistically significant differences could be demonstrated. This experiment, however, gave definite indications that the lower alkyl esters were affecting germination. Later experiments with longer exposure periods gave differences that were significant.

From the data obtained in these experiments it is apparent that aliphatic (1–5 carbon) esters of 2,4-dichlorophenoxyacetic acid are volatile enough to affect the germination of seeds which have been exposed to such vapors even though the seeds were air-dry. The data also indicate that the volatility of the 2,4-D esters varies and, in general, decreases as the length of the aliphatic carbon chain increases (see also fig. 2). By the same criteria the alkanolamine and sodium salts of 2,4-D are nonvolatile. The isopropyl ester of 2,4,5-trichlorophenoxyacetic acid also possesses sufficient volatility to affect the subsequent germination of air-dry seeds which have been ex-

TABLE 3

PERCENTAGE OF SEEDS GERMINATING IN 10 DAYS AFTER 60-DAY EXPOSURE TO VAPOR OF VARIOUS 2,4-D DERIVATIVES

Seeds	Untreated control	Derivatives of 2,4-dichlorophenoxyacetic acid							Isopropyl ester 2,4,5-T
		Na salt	Alkanolamine salt	Methyl ester	Ethyl ester	Isopropyl ester	n-butyl ester	n-amyl ester	
Tomato	65	82	75	0	6	16	49	57	56
Bean	86	98	88	58	62	82	94	88	62
Corn	94	100	94	88	88	88	80	79	84
Cucumber	90	94	98	0	12	66	84	90	68
Rye	40	31	32	0	0	2	11	24	3
Wheat	92	76*	96	22	41	72	74	86	77
Turnip	81	67	83	0	0	0	1	6	29
Cotton	26	30	23	1	1	5	26	26	25
Pea	98	96	94	2	2	8	52	78	78
Squash	68	98	88	0	0	2	6	54	4
Radish	79	70	64	0	0	0	5	50	40
Muskmelon	50	46	62	0	0	0	12	.....	10

* Mouse damage observed.

TABLE 4

PERCENTAGE OF CUCUMBER SEEDS GERMINATING IN 10 DAYS AFTER 180-DAY EXPOSURE TO VAPOR OF VARIOUS 2,4-D DERIVATIVES

Treatment	Percentage
Control	100
2,4-D acid	92
Triethanolamine salt	92
Alkanolamine salt	96
Sodium salt	100
Methyl ester	0

TABLE 5

PERCENTAGE OF CUCUMBER SEED GERMINATION. SEE TEXT FOR EXPLANATION

Treatment	Not soaked		Overnight soaking	
	Peeled	Not peeled	Peeled	Not peeled
Methyl ester	40	0	32	0
Control	72	80	92	92

posed to its vapors. In general, this effect is not so severe as that experienced with the isopropyl ester of 2,4-D.

A statistical treatment of the tomato, rye, turnip, and cucumber data of table 1 by analysis of variance indicated that the differences between the germination counts for the volatile and nonvolatile compounds were highly significant (odds = 1:99). In this analysis the results for the controls and the sodium and alkanolamine salts of 2,4-D were grouped and tested for significance against the results for the group composed of the methyl, ethyl, and isopropyl esters of 2,4-D. In table 3 the differences are of sufficient magnitude that their significance is assumed. In table 4 the difference between the methyl ester and the other 2,4-D derivatives is significant. The other differences between the treatments are not significant.

In the experiments in which the 2,4-D derivatives had obviously affected the seeds there were four types of effects noted on the germinating seeds: (*a*) the embryonic plants failed to break through the seed coats; (*b*) the seedlings emerged but failed to grow; this was usually accompanied by abnormal swellings on the seedling, and in this case the roots often did not develop properly; (*c*) leaf modification of the young leaves after emergence; leaves showed typical malformations such as are caused by 2,4-D when applied as a spray (fig. 2); (*d*) stunting of seedlings (fig. 3) and a slower germination of the seeds; this might or might not be accompanied by leaf modification.

Some of these effects are similar to these described by DUNLAP (1) for cotton seed obtained from plants affected by 2,4-D.

An interesting point about the delay in germination is that often it occurred initially in the germination process. After germination started, the rate was frequently about the same as that of the controls.

The effect on rates of germination is illustrated by the data plotted in figure 4 for peas exposed 30 and 60 days. Note that in the 30-day treatment the data for the amyl and butyl esters are not distinguishable from the control on the tenth day after planting. Nevertheless, the graph shows a 36–48-hour inhibition of germination. The data for the more volatile, lower alkyl esters (methyl, ethyl, isopropyl) show both an inhibition period and a depressed final count. Similar curves for rate of germination were obtained for the other seeds. For the 60-day exposure, rates of germination of the control seeds and those exposed to the nonvolatile compounds of 2,4-D are similar, while for those exposed to the esters the rates in general vary according to molecular weight. The volatility in such a series of compounds can normally be expected to vary inversely with molecular weight.

TABLE 6

VAPOR PRESSURE AT 80° F. (26.6° C.) OF ESTERS OF 2,4-DICHLOROPHENOXYACETIC ACID*

Ester	Vapor pressure (Mm. mercury)
Methyl	1.55×10^{-3}
Ethyl	0.86×10^{-3}
Isopropyl	1.2×10^{-3}

* For comparison, the vapor pressure of mercury at 80° F. is 2.1×10^{-3}.

From the data obtained with the peeled and unpeeled cucumber seeds (table 5) it seems that vapors from the volatile esters of 2,4-D do not penetrate the dry seed coats in a 180-day period. Whether the toxic material enters the seed with water during the imbibition of water in the germination process or

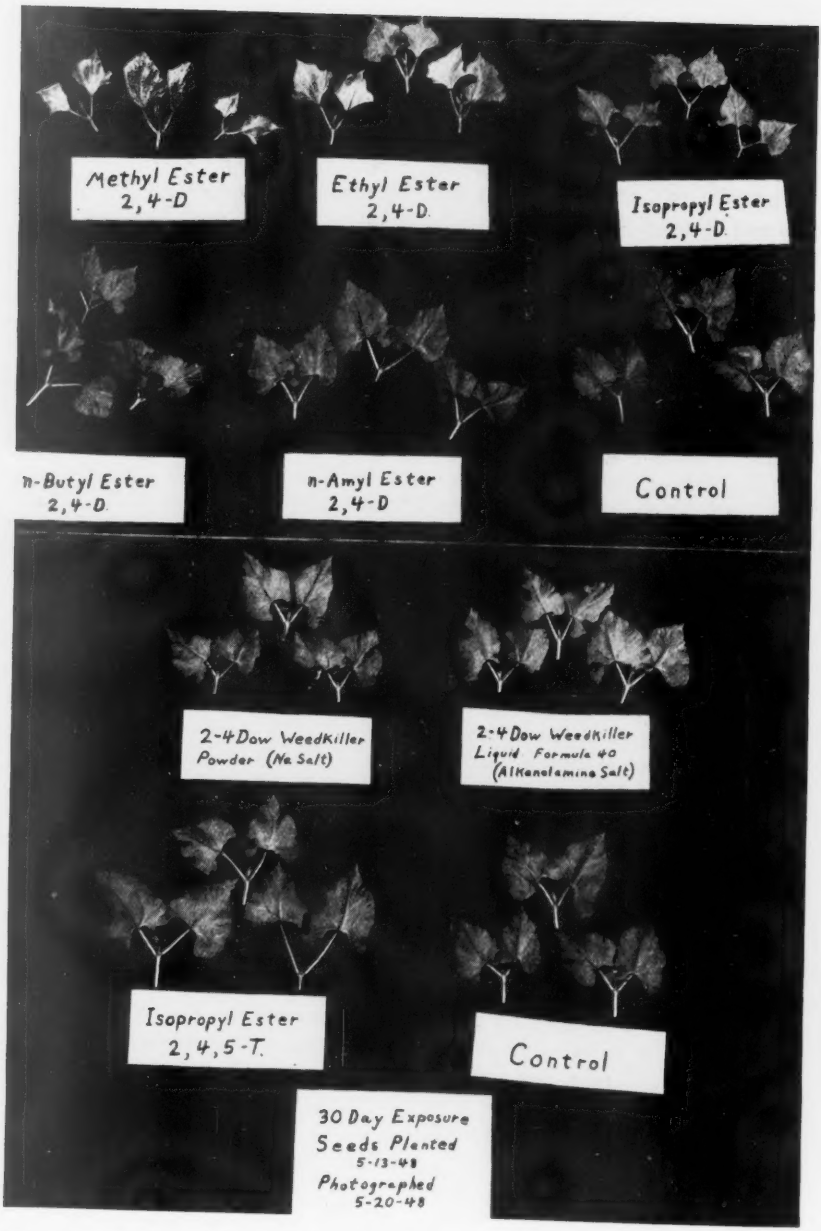


FIG. 2.—Primary leaves of bean seedlings from seeds exposed air-dry to vapors of derivatives of 2,4-D and 2,4,5-T. Note variation in modification induced by esters of 2,4-D and lack of effect of sodium and alkanolamine salts; also different effects of isopropyl esters of 2,4-D and 2,4,5-T.

whether it is absorbed by the young seedling as it emerges and breaks through the seed coat is not quite clear, although this experiment indicates that little 2,4-D enters the seed with water during the imbibition process.

There are considerable differences among species in the susceptibility of dry seeds to vapors from the volatile esters of 2,4-D. Some species such as corn are quite resistant, whereas others such as radishes or muskmelon are quite susceptible. On the other hand, cotton, which one might expect to be quite susceptible because of the extreme susceptibility of growing cotton to 2,4-D sprays, was more resistant than several other species such as turnips.

Previously reported work has shown that the sodium and alkanolamine salts of 2,4-D did not give off sufficient vapor in a confined space to affect growing

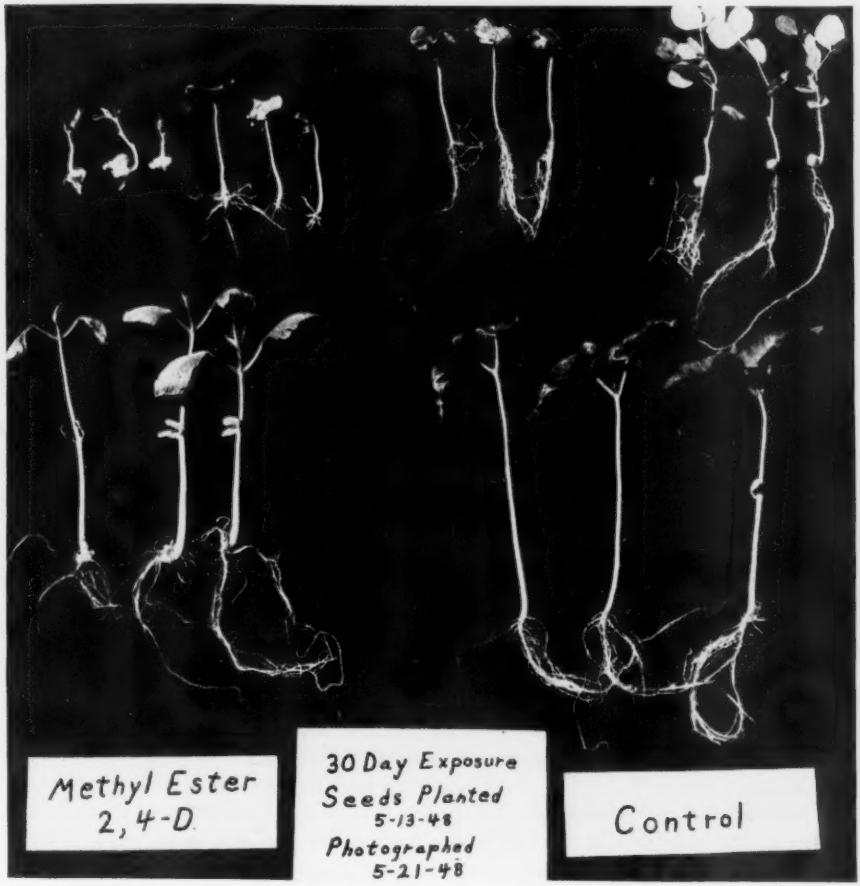


FIG. 3.—Pea and cotton seedlings (*above*) and bean seedlings (*below*) from control seeds and from seeds exposed air-dry to methyl ester of 2,4-D. Note leaf modification in beans, abnormal irregular swellings on pea roots, and stunting of cotton induced by chemical.

green plants. The present experiments give additional evidence that the sodium and alkanolamine salts of 2,4-D are nonvolatile in that the germination counts of air-dry seeds stored over these compounds in a confined atmosphere for periods up to 60 days were not noticeably depressed.

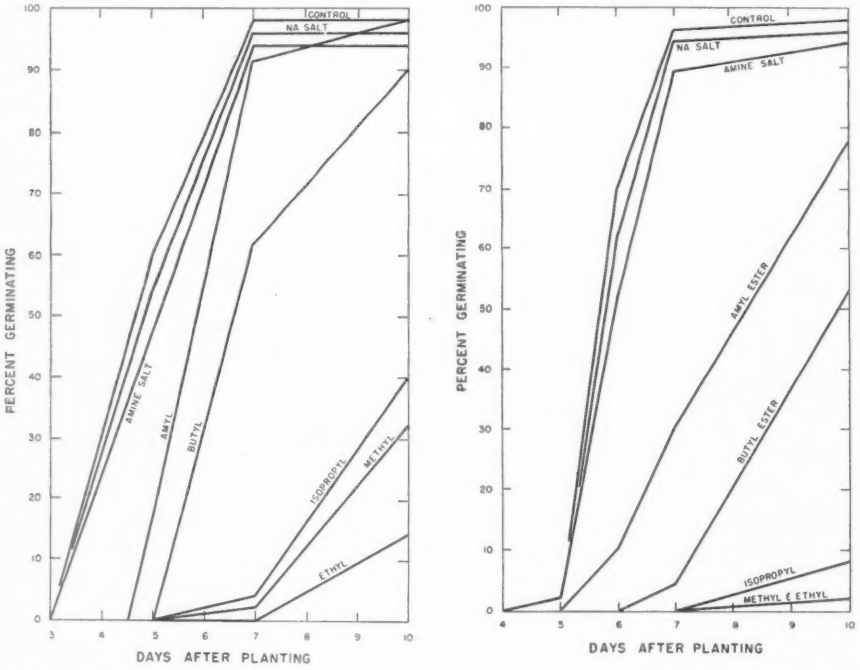


FIG. 4.—Rates of germination of pea seeds exposed air-dry to vapors of derivatives of 2,4-D for 30 days (*left*) and 60 days (*right*).

Summary and conclusions

1. Seeds should not be stored where they will be exposed to the vapors from volatile esters of 2,4-D.

2. Aliphatic (1–5 carbon) esters of 2,4-dichlorophenoxyacetic acid and the isopropyl ester of 2,4,5-trichlorophenoxyacetic acid are sufficiently volatile to affect adversely the germination of numerous species of field-crop and vegetable seeds exposed to such vapors. The degree of severity of the effect is proportional to the time of exposure.

3. The higher aliphatic esters (4–5 carbon) of 2,4-dichlorophenoxyacetic acid are less volatile than the lower esters (1–3 carbon) as determined by the effect of their vapors on the germination of seeds.

4. The sodium and alkanolamine salts of 2,4-dichlorophenoxyacetic acid are apparently not sufficiently volatile to affect the germination of air-dry seeds.

5. The ester vapor appears to be adsorbed on the seed coat and does not enter the dry seed before germination.

BIOCHEMICAL RESEARCH LABORATORY
DOW CHEMICAL COMPANY
MIDLAND, MICHIGAN

LITERATURE CITED

1. DUNLAP, A. A. 2,4-D injury to cotton from airplane dusting on rice. Phytopath. **38**:638–644. 1948.
2. MULLISON, W. R. The volatility of several salts and esters of 2,4-D as determined by the response of tomato, bean, and cotton plants. Proc. Amer. Soc. Hort. Sci. **53**: 281–290. 1949.
3. STATEN, G. Contamination of cotton fields by 2,4-D or hormone-type weed sprays. Jour. Amer. Soc. Agron. **38**:536–544. 1946.

EFFECT OF APPLICATION OF SUGAR ON THE TRANSLOCATION OF SODIUM 2,4-DICHLOROPHENOXYACETATE BY BEAN PLANTS IN THE DARK[1]

LAWRENCE M. ROHRBAUGH AND ELROY L. RICE

Introduction

Conditions affecting the translocation of growth-regulating substances have been studied by several workers. MITCHELL and BROWN (**2**) treated leaves of snap bean with 2,4-dichlorophenoxyacetic acid (2,4-D). They found that stem curvature, which was used as a criterion of translocation of the 2,4-D from a leaf to a stem, did not result when the plants were kept in a shaded location or in darkness. Very marked stem curvatures developed from similar treatments if the plants were kept in bright light. Similar results were obtained with Red Kidney bean plants by WEAVER and DEROSE (**8**). PENFOUND and MINYARD (**4**) treated water hyacinth and Red Kidney bean plants with the butyl ester of 2,4-D. They stated that the herbicide produced greater epinasty and greater necrosis in water hyacinth when the plants were kept in the shade than when they were exposed to full sunlight. They also claimed that the herbicide, when placed on one of the primary leaves of Red Kidney bean plants, effected similar bending and necrosis of the plants regardless of whether they were kept in darkness, in diffuse light, or in direct sunlight. RICE (**5**), using ammonium 2,4-dichlorophenoxyacetate [NH_4(2,4-D)] and Red Kidney bean plants, found no evidence that the herbicide was translocated from the leaves into the stems when the plants were in the dark. MITCHELL and BROWN (**2**) found that the translocation of 2,4-D was dependent on the presence of food materials in the leaves at the time translocation occurred. The 2,4-D was not translocated out of the leaves of plants when the sugar content of the leaves was low. WEAVER and DEROSE (**8**) found that, when the growth-regulator was applied to aerial portions of plants, it moved downward readily whenever translocation of synthesized materials occurred.

SPOEHR (**6**) showed that albino corn plants could absorb sufficient sugar through their leaves to increase greatly their length of life. WENT and CARTER (**9**) found that tomato plants could absorb significant amounts of sugar through their leaves. It occurred to the authors of the present paper that the application of sugar to the leaves of plants in the dark might affect their ability to translocate plant growth-regulators.

[1] This work was supported in part by a grant from the University of Oklahoma Faculty Research Committee.

Experimentation

Seeds of Red Kidney bean were planted (five seeds per pot) in 4-inch glazed pots and allowed to germinate on a well-lighted greenhouse bench. After emergence of the seedlings they were thinned to two plants per pot. When the first trifoliate leaves began to expand, ninety pots containing the most uniform plants were selected from a total of one hundred and thirty. These were transferred to a basement room, a portion of which could be completely darkened. The temperature in this room varied from 28° to 31° C. during the period of these experiments. The plants were then divided into three groups of thirty pots each, and each group was divided into two subgroups, subgroup A consisting of twenty pots and subgroup B of ten. Group 1 was placed under fluorescent lamps which produced light intensities ranging from 450 to 750 foot candles at the level of the plants. Groups 2 and 3 were placed in complete darkness. After 21 hours in the dark the leaves of the plants in group 2 were each dipped in a 10% solution of sucrose. The leaves were dipped again twice after intervening periods of 1 hour each. After 24 hours in the basement room (1 hour after the third and last dipping in sucrose of the leaves of the plants in group 2), 0.05 ml. of water containing 70 μgm. of sodium 2,4-dichlorophenoxyacetate [Na(2,4-D)] was applied as a drop to the upper surface of one of the primary leaves of each of the forty plants in subgroup A in each of the three groups. The twenty plants in sub-group B of each of the three groups were not treated with Na(2,4-D). A red light of low intensity was used while applying the sugar and growth-regulator to the plants in the dark.

Twenty-four hours after application of the growth-regulator, the treated primary leaf was removed from each plant by severing the base of the petiole with a razor blade. One primary leaf was similarly removed from each of the plants not treated with Na(2,4-D). The amount of stem curvature was measured with a transparent protractor, and all the plants were then moved to a well-lighted greenhouse bench. Ten days later the blades of the trifoliate leaves were removed from each plant and their fresh weight determined. The results are shown in table 1. This experiment was repeated with similar results.

In another experiment the tip of one of the primary leaves of each plant was cut off with a razor blade and the cut end of this leaf then placed in a vessel containing a 10% sucrose solution. A few hours later Na(2,4-D) was applied to the upper surface of this leaf in the manner described above. The cut end of the leaf was left in the sugar solution for 24 hours after the growth-regulator was applied, and the leaf was then removed and the plants were treated as in the earlier experiment. This method of application of sugar gave results quite similar to those obtained by dipping the leaves in the sugar solution.

The relative effects of three different sugars were studied in a later experiment. The seedlings were obtained and selected in the same way as described above and were transferred to the darkroom when the first trifoliate leaves had begun to expand. The plants were divided into four groups. Groups 4, 5, and 6 contained two subgroups of twenty plants each. Group 7 contained twenty-four plants. After 22 hours in the dark the plants were treated as described below.

The tip of one primary leaf of each plant in group 4 was removed with a razor blade, and the cut end was oriented to dip into about 15 ml. of 10% sucrose

solution in a Syracuse watch glass. About 1 hour later, 0.05 ml. of water containing 100 μgm. of Na(2,4-D) was applied as a drop to the upper surface of the leaf in the sugar solution on each of the plants in subgroup A. The growth-regulator was placed on the midrib near the base of the leaf. No Na(2,4-D) was placed on the leaves of the plants in subgroup B. The ends of the leaves were left in the sugar solution until the next day.

The plants in groups 5 and 6 were treated in the same way as those in group 4 except that 10% fructose or glucose solutions were used, respectively, in place of the sucrose solution. In each group the plants in subgroup A were treated with Na(2,4-D), but those in subgroup B were not.

The plants in group 7 were left in the dark for the same length of time as those in the preceding groups, but they were not treated with sugar or with the growth-regulator.

Twenty-four hours after application of the growth-regulator, the primary leaves which had been in the sugar solutions (and one of the primary leaves of each plant in group 7) were removed, and the stem curvature of each plant was measured. The plants were then returned to the greenhouse as in the earlier experiments. Nine days later the blades of the trifoliate leaves were removed from each plant and weighed. The data appear in table 2.

TABLE 1

EFFECT OF NA(2,4-D) ON STEM CURVATURE AND WEIGHT OF TRIFOLIATE LEAVES OF BEAN PLANTS TREATED IN LIGHT AND IN DARK WITH AND WITHOUT APPLICATION OF SUCROSE

Group and Subgroup	Treatment	No. of plants	Mean stem curvature (°)	Mean weight of trifoliate leaves (gm.)
1	In light			
A	70 μgm. Na(2,4-D)	40	31.0	0.238±0.033*
B	No Na(2,4-D)	20	0	0.480±0.054*
2	In dark with sucrose			
A	70 μgm. Na(2,4-D)	40	20.4	0.453±0.037†
B	No Na(2,4-D)	20	0	0.593±0.048†‡
3	In dark without sucrose			
A	70 μgm. Na(2,4-D)	40	0	0.467±0.040
B	No Na(2,4-D)	20	0	0.460±0.038‡

* Difference between subgroups 1A and 1B significant at 1% level.
† Difference between subgroups 2A and 2B significant at 2% level.
‡ Difference between subgroups 2B and 3B significant at 1% level.

Results and Discussion

Sodium 2,4-dichlorophenoxyacetate was translocated out of the primary leaves of destarched bean plants in the dark when sugar solutions were applied to the leaves. This was evidenced by the curvature of the stems and the inhibition of growth of the trifoliate leaves of treated plants. Stem curvature has been used as evidence of translocation of growth-regulating substances by several investigators (**1, 2, 5, 8**). Inhibition of growth of terminal buds and trifoliate leaves has also been used as evidence of this process

(3, 5, 7). There was no indication in any of these experiments that Na(2,4-D) was translocated out of destarched leaves in the dark unless sugar was applied to the leaves. This is in agreement with the findings of MITCHELL and BROWN (2) and a number of other workers.

If stem curvature and inhibition of the growth of the trifoliate leaves are used as indexes of the amount of growth-regulator translocated, the data presented in table 2 indicate that fructose and glucose were more effective in promoting the translocation of Na(2,4-D) than was sucrose. The use of either of these hexoses resulted in inhibitions of growth which were significantly greater than that produced by the use of sucrose. The order of the relative effects of the three sugars as evidenced by stem curvature was the same as that based on leaf inhibition. The stem curvatures, however, resulting from treatment with the different sugars were not significantly different. RICE (5) found that stem curvatures were often not statistically correlated with the amount of NH_4(2,4-D) absorbed and that inhibition of the growth of the trifoliate leaves was a better index of the amount of the growth-regulator absorbed than was the degree of stem curvature.

TABLE 2

EFFECT OF APPLICATION OF DIFFERENT SUGARS ON STEM CURVATURE AND WEIGHT OF TRIFOLIATE LEAVES OF BEAN PLANTS TREATED WITH Na(2,4-D) IN THE DARK

Group and Subgroup	Treatment	No. of plants	Mean stem curvature (°)	Mean weight of trifoliate leaves (gm.)
4	Ends of leaves in sucrose solution			
A	100 μgm. Na(2,4-D)	20	33.0	0.499 ± 0.064*
B	No Na(2,4-D)	20	0.5	0.845 ± 0.042
5	Ends of leaves in fructose solution			
A	100 μgm. Na(2,4-D)	20	46.5	0.199 ± 0.039*
B	No Na(2,4-D)	20	0.5	0.898 ± 0.049
6	Ends of leaves in glucose solution			
A	100 μgm. Na(2,4-D)	20	44.0	0.274 ± 0.047
B	No Na(2,4-D)	20	3.2	0.874 ± 0.046
7	No sugar or Na(2,4-D) applied	24	0.0	0.933 ± 0.052

* Differences between subgroups 4A and 5A and between subgroups 4A and 6A significant at 1% level.

Summary

1. A drop of an aqueous solution of sodium 2,4-dichlorophenoxyacetate (containing 70–100 μgm.) was applied to the upper surface of one of the primary leaves of each of a number of Red Kidney bean plants, some of which were kept in the light before and after application of the growth-regulator and some of which were kept in the dark for 24 hours before and after treatment. The leaves of some of the plants in the dark were dipped in sucrose solution several times shortly (1–3 hours) before the growth-regulator was applied. Sugar was supplied to still other plants by placing the cut end of one

of the primary leaves of each plant in a vessel containing a 10% sugar solution.

2. The curvatures of the stems were measured 24 hours after application of the herbicide, and the treated leaves were removed at this time. The plants were then placed in the greenhouse for 10 days, at the end of which time the weight of the trifoliate leaves from each plant was determined.

3. No evidence was found that Na(2,4-D) was translocated out of destarched bean leaves in the dark unless sugar was added to the leaves. The application of sugar to the leaves of destarched plants which were subsequently treated with Na(2,4-D) resulted in stem curvature and inhibition in growth of the trifoliate leaf blades. Such effects were not apparent when sugar was not applied.

4. Fructose and glucose were significantly more effective than sucrose in augmenting the translocation of the growth-regulator.

DEPARTMENT OF PLANT SCIENCES
UNIVERSITY OF OKLAHOMA

LITERATURE CITED

1. ENNIS, W. B., JR., and BOYD, F. T. The response of kidney-bean and soybean plants to aqueous-spray applications of 2,4-dichlorophenoxyacetic acid with and without Carbowax. BOT. GAZ. **107**: 552–559. 1946.

2. MITCHELL, J. W., and BROWN, J. W. Movement of 2,4-dichlorophenoxyacetic acid stimulus and its relation to the translocation of organic food materials in plants. BOT. GAZ. **107**:393–407. 1946.

3. MITCHELL, J. W.; WOOD, J. W.; WOLFE, W. C.; and IRVING, G. W. Relative growth rates of bean and oat plants containing known amounts of a labeled plant-growth regulator (2-iodo^{131}-3-nitrobenzoic acid). Science **106**:395–397. 1947.

4. PENFOUND, W. T., and MINYARD, VIRGINIA. Relation of light intensity to effect of 2,4-dichlorophenoxyacetic acid on water hyacinth and kidney-bean plants. BOT. GAZ. **109**:231–234. 1947.

5. RICE, E. L. Absorption and translocation of ammonium 2,4-dichlorophenoxyacetate by bean plants. BOT. GAZ. **109**:301–314. 1948.

6. SPOEHR, H. A. The culture of albino maize. Plant Physiol. **17**:397–410. 1942.

7. WEAVER, R. J. Effect of spray applications of 2,4-dichlorophenoxyacetic acid on subsequent growth of various parts of Red Kidney bean and soybean plants. BOT. GAZ. **107**:532–539. 1946.

8. WEAVER, R. J., and DEROSE, H. R. Absorption and translocation of 2,4-dichlorophenoxyacetic acid. BOT. GAZ. **107**:509–521. 1946.

9. WENT, F. W., and CARTER, MARCELLA. Growth response of tomato plants to applied sucrose. Amer. Jour. Bot. **35**:95–106. 1948.

PRELIMINARY ATTEMPTS IN LARCH EMBRYO CULTURE

CLARENCE STERLING[1]

Introduction

In describing embryogeny in the Pinaceae, BUCHHOLZ (**2**) showed that the embryos of a certain group of genera (*Pinus*, *Cedrus*, *Tsuga*) undergo cleavage regularly. On the other hand, another group (*Picea*, *Abies*,[2] *Larix*, *Pseudotsuga*) usually has simple polyembryony (without cleavage). These generic distinctions have been assigned a certain phylogenetic significance by BUCHHOLZ, a point which was discussed and challenged by THOMSON (**12**). THOMSON suggested that these ontogenetic differences may not be invariable in the embryos but might rather reflect differences in nutritional conditions in the female gametophyte. The present investigation was initiated in order to test THOMSON's suggestion.

Material and methods

Ovulate cones of *Larix decidua* Mill. were collected June 10–16, 1948, and the ovules removed. The gametophytes were dissected out, immersed in 0.1% $HgCl_2$ solution for 3–5 minutes, and then rinsed several times in sterile distilled water. Embryo systems were dissected from the gametophytes in a sterile solution of 10% sucrose and removed to the culture tubes with a flamed pipette.

For culture media, variations of the solutions used by LOO and WANG (**6**) were tried. The four basal culture media consisted of an aqueous solution of mineral salts, modified after WHITE (**10**); the following vitamins (in mg. per liter): nicotinic acid (1.0), B_1 (0.5), B_6 (0.25), ascorbic acid (20.0), calcium pantothenate (1.0), and riboflavin (0.3); and sucrose in the respective concentrations (gm. per liter): 20, 50, 100, and 150. In addition, series of cultures were set up in which glutamic acid and cysteine hydrochloride (**3**) (3.0 mg. per liter of each), indoleacetic acid (0.01 mg. per liter), and acid-hydrolyzed casein (**5**) (0.84%, adjusted to approximately pH 5.2 with normal NaOH) were added singly and in combination to the basal media. Finally, two additional culture series combined the four basal media with higher concentrations of indoleacetic acid (0.1 and 1.0 mg. per liter).

These combinations resulted in forty separate culture media, which were made up with 1% agar for slanting in test tubes. Five tubes, with 10 cc. each, were prepared from each medium and sterilized for 20 minutes at 15 pounds pressure. Two to five embryos were placed in each tube and kept at room temperature for a period of 2 months. At the conclusion of the experiment samples were taken from each series and prepared as whole mounts in glycerine jelly.

Observations

The youngest embryos placed in culture had fairly long primary suspensor cells. Usually the embryonic cells had produced two tiers of unelongated em-

[1] This study was supported in part by a grant from the University Research Committee of the Graduate School, with funds supplied by the Wisconsin Alumni Research Foundation.

[2] There may be a limited percentage of cleavage among embryos of *Abies balsamea* (**1**, **4**).

bryonal tubes. In the largest embryos, the embryonal tubes were elongating, and there were about eight tiers of cells at the apex.

On trees growing on the campus the embryos developed normally, reaching full size in a period of about 5–6 weeks after fertilization. After 4 weeks, when most embryos in culture had increased only slightly in size and had ceased to grow, many embryos on the tree were generally over 3 mm. long and had already produced cotyledons. On the whole, relatively few embryos (about 15% of the total) grew to any extent in culture. Hence, the number which grew in each medium is too small to correlate development directly with the composition of the medium.

Sample embryos raised in culture are shown in figures 1–34. In figure 1 there seems to be a normally formed embryo with its suspensor. This resemblance, however, is superficial, the internal structure here consisting of a cordon of elongated cells surrounded by a mass of irregularly arranged, isodiametric cells. As observed at weekly intervals, this embryo grew first as a multicellular cylinder of tissue, composed of elongated cells. After about 30 days, enlargement took place at the tip to produce the widened mass of cells shown. Growth had ceased at least 2 weeks before the embryo was removed from culture.

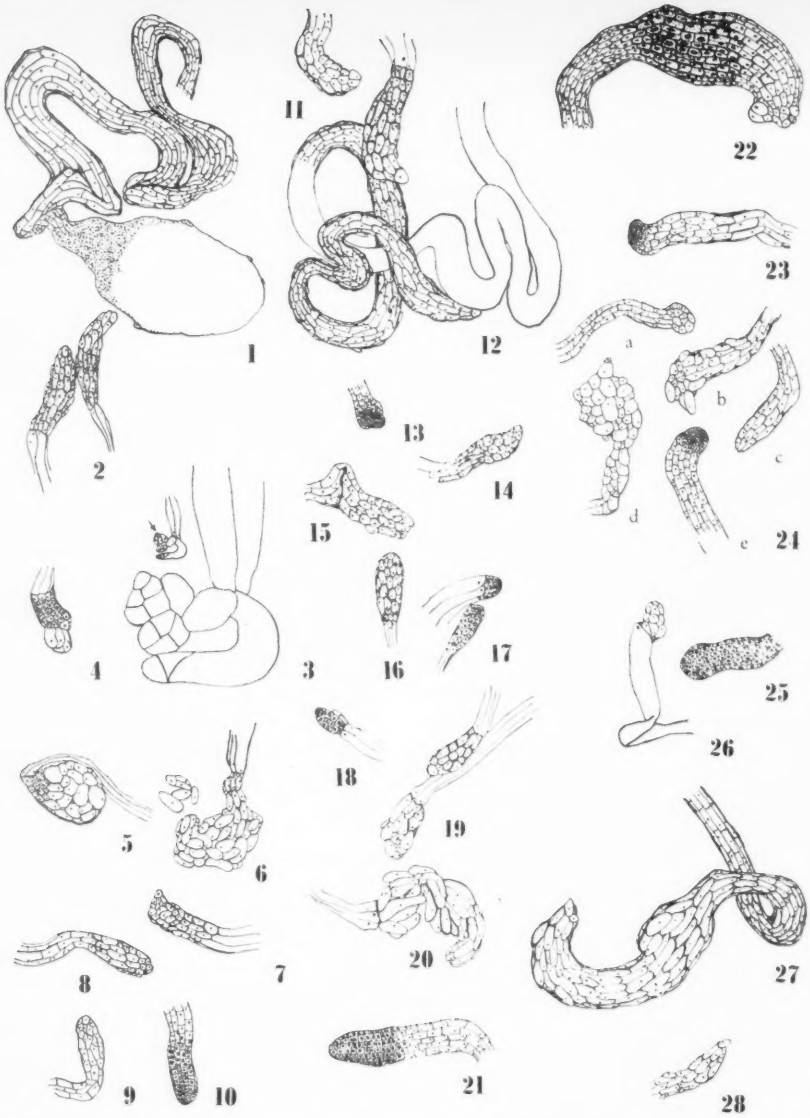
In figure 12 an embryo with a particularly large number of cells has been produced as a suspensor-like body which has curved continually and doubled back on itself during growth. Unlike the embryo of figure 1, no enlargement has taken place at the tip. Similar embryos are seen in figures 22 and 27, four embryos like that of the latter being produced in the same medium. In connection with the suspensor-like growths, it is interesting that some embryos experienced a limited development which seems to have been typically embryonic, with a paraboloid mass of densely staining cells at the end of the suspensor (figs. 10, 21, 24, 25, 30).

A particularly noteworthy feature of embryonic growth in culture is the fact that three cases of cleavage were found in the experiment. One occurred at a sucrose concentration of 50 gm. per liter in the basal medium (figs. 32, 33) and another in a sugar concentration of 100 gm. per liter in the same medium (fig. 3). Cleavage here involved the separation of the first embryonal tubes at the tip of the four primary suspensor cells. In both cases growth apparently stopped soon after the cleavage. In a different medium a third instance of cleavage was found (fig. 26), with a larger embryonic group attached to the tip of what appears to be a primary suspensor cell.

Other embryos in culture showed one of the very common phenomena demonstrated throughout the series: cell enlargement, formation of large vacuoles, and partial separation of previously embryonic cells (figs. 4–6, 19, 20, 24*b–d*, 29, 34). This phenomenon may be related to the occurrence of cleavage.

Discussion

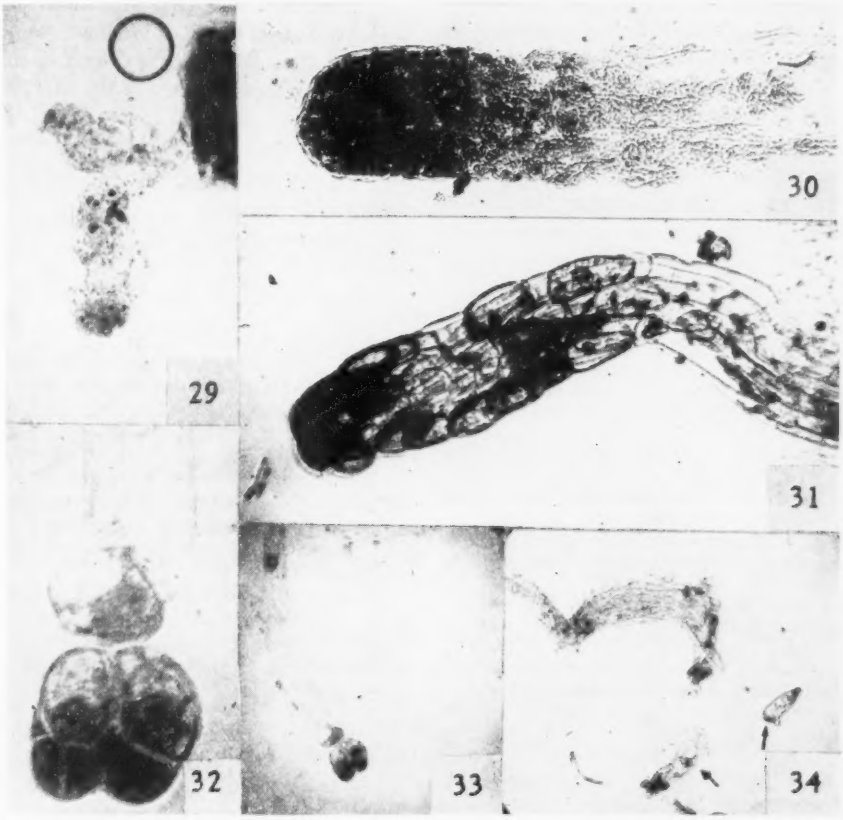
Since few embryos grew noticeably and since (as also noted by Loo and Wang) growth ceased early, it is difficult to make definite suggestions based on the composition of the medium. Future work should be concerned with perfection of sterilization and inoculation techniques and perhaps with a search for some material(s) initially supplied by the gametophyte ("embryo factor" [7, 8]), which may be lacking in the media of Loo and Wang and of the writer. Only in one embryo (from the medium with 0.1 mg. of



FIGS. 1–28.—Whole mounts of larch embryos grown *in vitro*. Figs. 1, 2, from culture with basal medium and 50 gm. sugar per liter. Figs. 3–6, from basal medium with 100 gm. sugar per liter; fig. 3 inset shows true relative size of embryo at arrow; fig. 6 with some cells loosened in mounting. Figs. 7, 8, from basal medium plus cysteine, glutamic acid, and casein, with 50 gm. sugar per liter. Figs. 9, 10, from basal medium and added casein and indoleacetic acid, 50 gm. sugar per liter. Figs. 11, 12, embryos from basal medium with glutamic acid, cysteine, and 50 gm. sugar per liter. Figs. 13–15, from basal medium with casein and 20 gm. sucrose per liter. Figs. 16, 17, embryos from basal medium, casein, and 50 gm. sugar per liter. Figs. 18–20, from basal medium, glutamic acid, cysteine, indoleacetic acid (0.01 mg. per liter), and 100 gm. sucrose per liter. Fig. 21, from same medium as in figs. 18–20, but with 150 gm. sucrose per liter. Figs. 22–26, from basal medium plus casein, cysteine, glutamic acid, and 0.01 mg. per liter of indoleacetic acid; fig. 22 with 50 gm. sugar per liter; fig. 23 with 20 gm. sugar per liter; fig. 24 *a–e* with 100 gm. sugar per liter; figs. 25, 26 with 150 gm. sugar per liter. Fig. 27, from basal medium, 0.1 mg. of indoleacetic acid per liter, and 20 gm. sucrose per liter. Fig. 28, as in fig. 27, but with 50 gm. sugar per liter. Fig. 3 ×150. All other figures ×30.

indoleacetic acid per liter and 50 gm. sucrose per liter) did growth continue beyond the 2-month period of culture. In this instance growth was uniform on all surfaces, as is characteristic of callus tissues. The anomalous growth of the embryos may be related to the physical as well as to the chemical composition of the different media.

The main feature of this investigation is its demonstration that cleavage can be induced in the larch. In intensive studies of the larch embryo *in vivo* (**2, 9**), cleavage has not been observed. Consequently, it appears significant that such cleavage can occur in certain synthetic media. The induction of cleavage in larch and the variable occurrence of cleavage nor-



FIGS. 29–34.—Photomicrographs of larch embryos grown *in vitro*. Fig. 29, embryo from basal medium and 0.1 mg. indoleacetic acid per liter plus 100 gm. sugar per liter. ×55. Fig. 30, embryo showing almost same structure as normal embryo, from medium with casein, 0.01 mg. indoleacetic acid per liter, and 50 gm. sugar per liter. ×108. Fig. 31, embryo from medium with glutamic acid, cysteine, casein, and 50 gm. sugar per liter. ×143. Fig. 32, embryo at tip of suspensor in basal medium with 50 gm. sugar per liter. ×260. Fig. 33, from same system as in fig. 32, showing another embryonic unit (single cell at tip of suspensor). ×55. Fig. 34, embryo from basal medium with 0.1 mg. indoleacetic acid per liter and 20 gm. sugar per liter. Arrows point to cells dislodged in mounting. ×21.

mally in *Abies* and *Taxus* (11) appear to confirm THOMSON's (12) suggestion that cleavage is less an invariable characteristic of the embryo than a function of nutritional control.

It must be indicated, however, that the normal nutrition of the embryo likewise has evolved with the species. Although these pinaceous embryos may have a latent potentiality for cleavage, each species has provided a certain material composition of the gametophyte which evokes a characteristic response in the embryo. Nevertheless, THOMSON's point remains valid: the intrinsic behavior of the embryo, presumably being susceptible to environmental alteration, perhaps may not be assigned the definite phylogenetic significance proposed by BUCHHOLZ.

Summary

Embryos of *Larix decidua* were placed on synthetic culture media at the stage at which the primary suspensors (and occasionally also the first embryonal tubes) were elongating. Usually several additional cells, destined to form embryonal tubes, were also present behind the apical embryonic tier. After 2 months, growth in the forty different media was limited in comparison with that in natural conditions. Not all embryos grew uniformly, and the type of growth was anomalous. In several cases cleavage of the first embryonal tubes occurred at the tips of the primary suspensors. The significance of this "abnormal" cleavage in the larch embryo is discussed.

DEPARTMENT OF BOTANY
UNIVERSITY OF WISCONSIN

LITERATURE CITED

1. BUCHHOLZ, J. T. Origin of cleavage polyembryony in conifers. BOT. GAZ. **81**: 55–71. 1926.
2. ———. The pine embryo and the embryos of related genera. Trans. Ill. Acad. Sci. **23**:117–125. 1931.
3. GAUTHERET, R. J. La culture des tissus. Gallimard, Paris. 1945.
4. HUTCHINSON, A. H. Embryogeny of *Abies*. BOT. GAZ. **77**:280–289. 1924.
5. KENT, N., and BRINK, R. A. Growth *in vitro* of immature *Hordeum* embryos. Science **106**:547–548. 1947.
6. LOO, S. W., and WANG, F. H. The culture of young conifer embryos *in vitro*. Science **98**:544. 1943.
7. OVERBEEK, J. VAN; CONKLIN, M. E.; and BLAKESLEE, A. F. Cultivation *in vitro* of small *Datura* embryos. Amer. Jour. Bot. **29**:472–477. 1942.
8. OVERBEEK, J. VAN; SIU, R.; and HAAGEN-SMIT, A. J. Factors affecting the growth of *Datura* embryos *in vitro*. Amer. Jour. Bot. **31**: 219–224. 1944.
9. SCHOPF, J. M. The embryology of *Larix*. Ill. Biol. Monographs **19**(4). Univ. of Ill. Press, Urbana. 1943.
10. SKOOG, F., and TSUI, C. Chemical control of growth and bud formation in tobacco stem segments and callus cultured *in vitro*. Amer. Jour. Bot. **35**:782–787. 1948.
11. STERLING, C. Proembryo and early embryogeny in *Taxus cuspidata*. Bull. Torrey Bot. Club **75**: 469–485. 1948.
12. THOMSON, R. B. "Polyembryony": sexual and asexual embryo initiation and food supply. Trans. Roy. Soc. Canada **39**(V):143–169. 1945.